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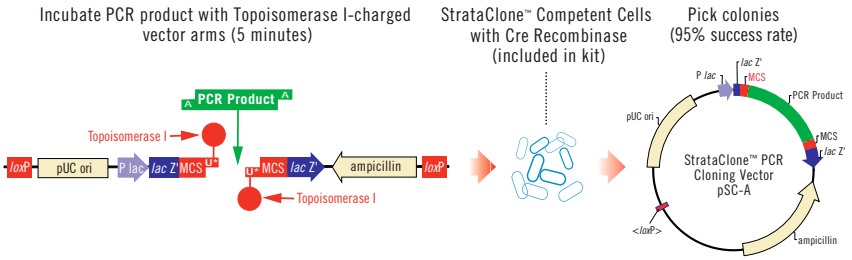

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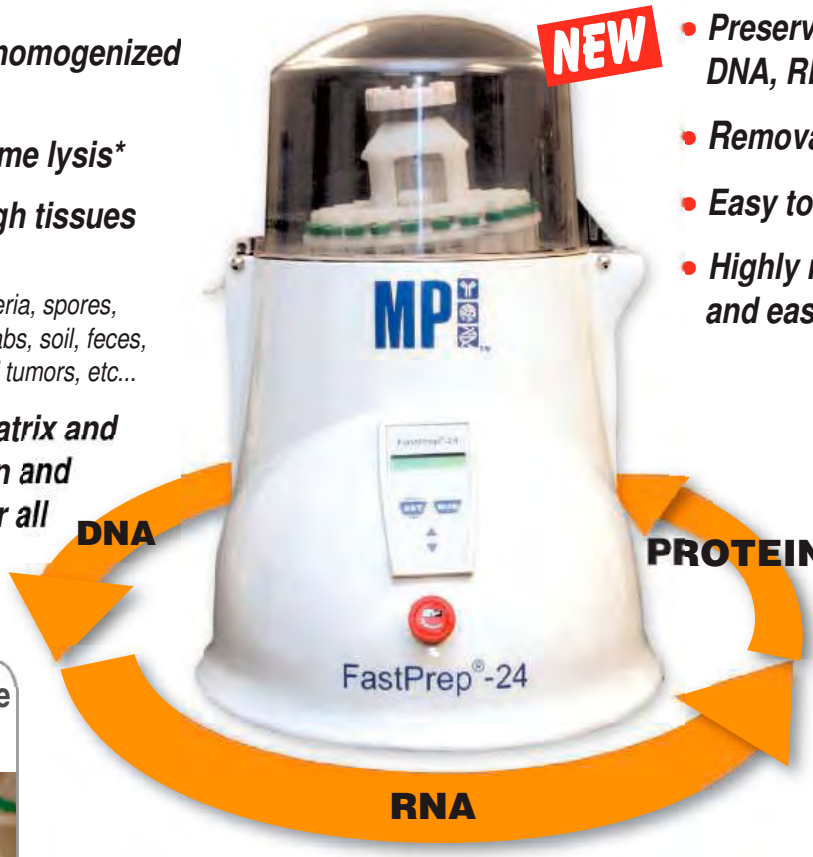

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Volume 315, Issue 5809

COVER

Scanning electron micrograph of *Trichomonas vaginalis* parasites (gray-green) adhering to vaginal epithelial cells (pink). Attached parasites are flattened and amoeba-like; parasites that do not adhere are pear-shaped. See page 207.

Image: Antonio Pereira-Neves and Marlene Benchimol, Santa Ursula University, Rio de Janeiro

DEPARTMENTS

EDITORIAL

NEWS OF THE WEEK

NEWS FOCUS

176

LETTERS

BOOKS *ET AL.*

190

POLICY FORUM

PERSPECTIVES

CONTENTS continued >>

Science

CONTENTS

SCIENCE EXPRESS

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MATHEMATICS

Clustering by Passing Messages Between Data Points
B. J. Frey and D. Dueck
An algorithm that exchanges messages about the similarity of pairs of data points speeds identification of representative examples in a complex data set, such as genes in DNA data.
10.1126/science.1136800

CHEMISTRY

Improved Oxygen Reduction Activity on $Pt_3Ni(111)$ via Increased Surface Site Availability
V. R. Stamenkovic et al.
The Pt-enriched outer surface layer of the close-packed (111) surface has an altered electronic structure that favors O_2 adsorption over species such as OH.
>> News story p. 172
10.1126/science.1135941

EVOLUTION

BREVIA: Floral Gigantism in Rafflesiaceae
C. C. Davis, M. Latvis, D. L. Nickrent, K. J. Wurdack, D. A. Baum
Rafflesiaceae plants with huge flowers but neither stems nor leaves have been evolutionarily mysterious; they are now shown to be spurges (Euphorbiaceae).
10.1126/science.1135260

CHEMISTRY

Ex Situ NMR in Highly Homogeneous Fields: ^1H Spectroscopy
J. Perlo, F. Casanova, B. Blümich
A movable array of permanent magnets can produce a homogeneous magnetic field anywhere, allowing portable nuclear magnetic resonance spectroscopy at high resolution.
10.1126/science.1135499

TECHNICAL COMMENT ABSTRACTS

REVIEW

226

BREVIA

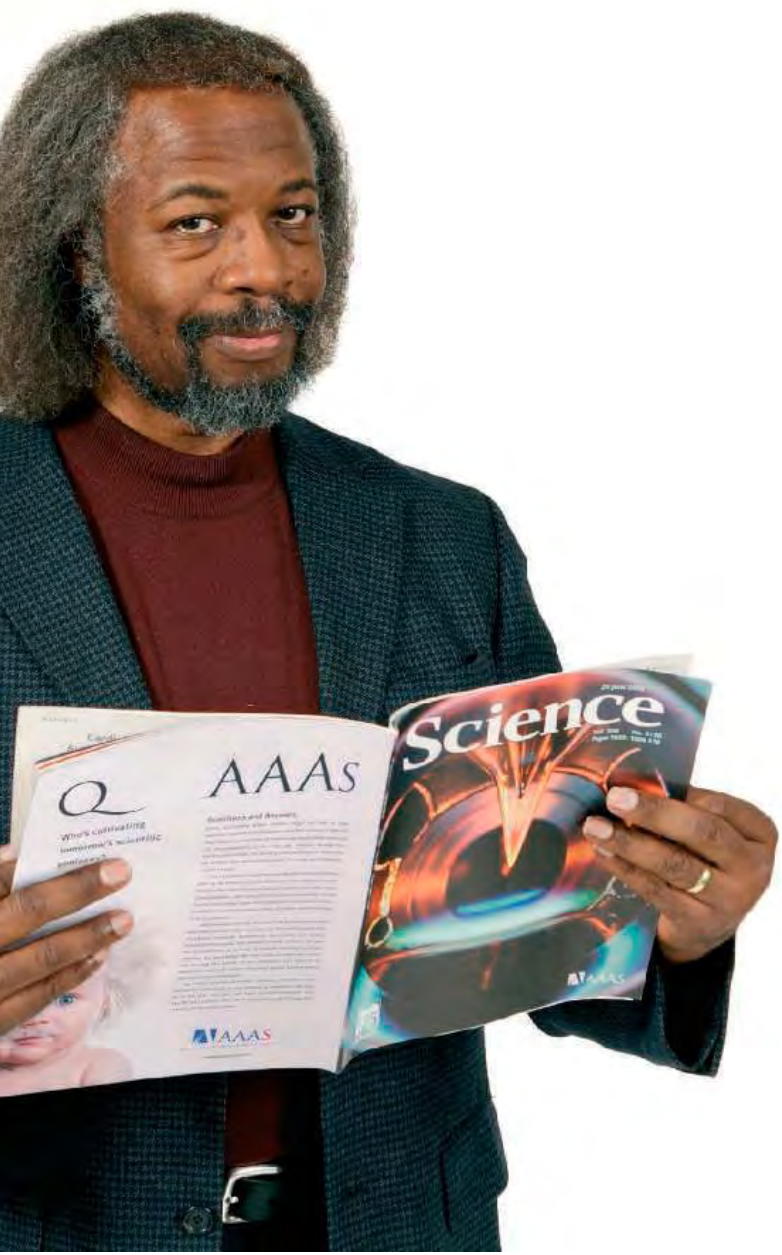
RESEARCH ARTICLE

REPORTS

CONTENTS continued **>>**

Q AAAs

Who's helping bring the gift of science to everyone?



"As a child I got very interested in space travel. When I was six my father gave me some books on rockets and stars. And my universe suddenly exploded in size because I realized those lights in the sky I was looking at were actually places.

I wanted to go there. And I discovered that science and technology was a gift that made this possible. The thrill of most Christmas presents can quickly wear off. But I've found that physics is a gift that is ALWAYS exciting.

I've been a member of AAAS for a number of years. I think it's important to join because AAAS represents scientists in government, to the corporate sector, and to the public. This is very vital because so much of today's science is not widely understood.

I also appreciate getting *Science* because of the breadth of topics it covers. It gives me a great grounding for many activities in my professional life, such as advising government agencies and private corporations."

Jim Gates is a theoretical physicist and professor at the University of Maryland. He's also a member of AAAS.

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S. James Gates Jr., Ph.D.
Theoretical physicist
and AAAS member

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REPORTS *CONTINUED...*

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CONTENTS continued **>>**

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P. Fiske
For grad students and postdocs, sometimes it's a good idea to color outside the lines.

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M. Bak-Maier and S. Inger
Collaborative, interdisciplinary projects can be hard to get off the ground.

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A. Kotok
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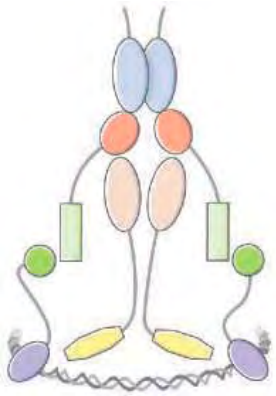

Mammalian Ssdp-Ldb transcription complex.

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PERSPECTIVE: Proline-Rich Regions in Transcriptional Complexes—Heading in Many Directions
V. Neduva and R. B. Russell
Proteins swap domains to preserve overall organization of a transcriptional complex.

GLOSSARY
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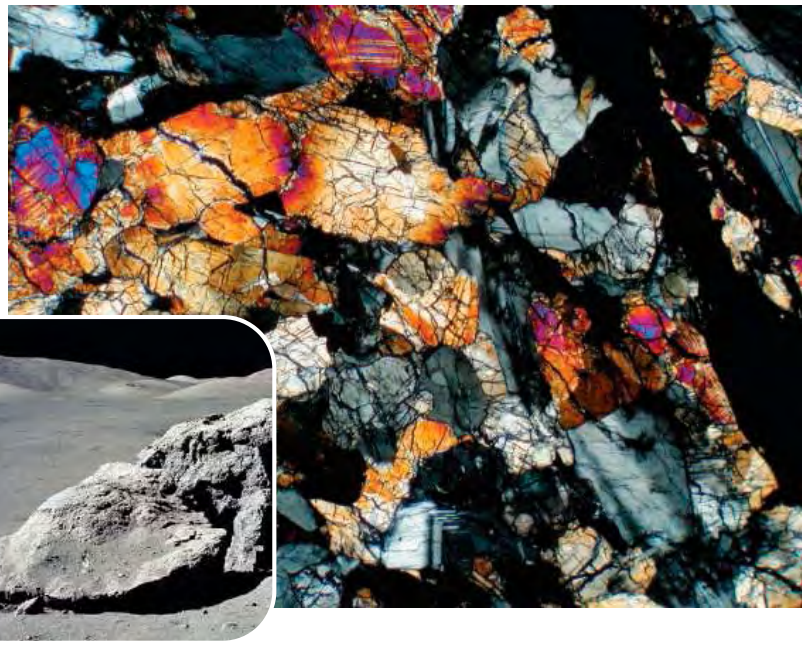
EDITED BY STELLA HURTLEY AND PHIL SZUROMI

Lunar Differentiation >>

Highly siderophile elements (HSE) are concentrated in Earth's core and depleted in its silicate mantle, but little is known about their lunar distribution. **Day *et al.*** (p. 217) present Re-Os isotope and HSE abundance data for lunar basalts which indicate that the lunar mantle has chondritic HSE ratios similar to Earth's silicate mantle, but with absolute abundances that are 20 times lower. Thus, the silicate-metal equilibration accompanying core formation must have depleted the HSEs in the silicate mantle of both the Moon and Earth, and continued accretion of meteoritic material replenished their mantles with HSEs. However, this late accretion must have terminated earlier on the moon than Earth, and is likely related to sealing of the lunar mantle by crust formation at or before 4.4 billion years ago.

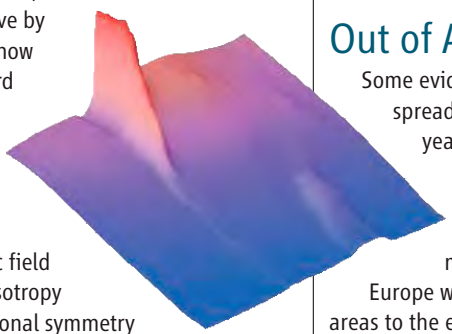


Supernova Shapes

Type Ia supernovae are widely used "standard candles" for distance measurements. **Wang *et al.*** (p. 212, published online 30 November; see the Perspective by **Leonard**) have collected spectra in polarized light of 17 such supernovae to investigate the geometry of these explosions. More powerful detonations produced more spherical ejecta, and the outer ejecta layers are more inhomogeneous than inner ones. These findings constrain the physics of burning in the supernovae and tightens the luminosity relations of type Ia supernovae that are used for cosmological measurements.

A Nematic Fermi Liquid

Previous work on strontium ruthenate has revealed the existence of a quantum critical point where the phase transition is driven by magnetic fields. **Borzi *et al.*** (p. 214, published online 23 November; see the Perspective by **Fradkin *et al.***) show that easy and hard directions for current flow can be observed that depend on the direction of the applied magnetic field and that this anisotropy breaks the tetragonal symmetry of the underlying crystal structure. The authors argue that their results are consistent with a recently predicted quantum phase of matter, a nematic Fermi liquid, and may present a test bed to explore other such exotic phases observed in other quantum systems where electronic correlation dominates.



Stability from a Gold Coat

A major problem for fuel cells in automotive applications is the tendency of the oxygen-reducing platinum cathode to dissolve during the repeated potential cycling required for braking and acceleration. **Zhang *et al.*** (p. 220; see the news story by **Service**) found that nanometer-scale gold clusters deposited on carbon-supported platinum particles effectively inhibit dissolution during electrochemical cycling experiments in a perchloric acid electrolyte. Surprisingly, the gold does not significantly inhibit the catalytic O_2 reduction, despite the low activity of gold alone in this reaction. X-ray absorption near-edge spectroscopic studies suggest that the presence of gold raises the platinum oxidation potential.

Out of Africa When?

Some evidence implies that modern humans spread out from Africa some 50,000 years ago and reached central and western Europe about 40,000 years ago. The colonization of northern Europe and Asia has been more difficult to date; northwestern Europe was covered in ice, but the land areas to the east were more open but still frigid (see the Perspective by **Goebel**). **Anikovich *et al.*** (p. 223) now show through a comparison of radiocarbon and luminescence dating and paleomagnetic data that a paleolithic archaeological site on the Don River, Russia (about 400 miles south of Moscow) dates to about 45,000 years ago. Although there are many fossils from this time scattered throughout Europe and Asia, ones from Africa for comparison and to test this hypothesis are scarce. **Grine *et al.*** (p. 226) have dated a skull first discovered in 1952 from Hofmeyr, South Africa, to about 36,000 years ago based on luminescence data of attached quartz. The skull displays several features that are more primitive than contemporaneous European skulls but is consistent with the emergence of modern humans from sub-Saharan Africa.

Interference in the Secondary

The effector molecules in RNA interference (RNAi) are small interfering (si)RNAs. The initial population of "primary" siRNAs, ~22-nucleotides in length with 5′-monophosphates groups, is generated by the Dicer nuclease. Amplification and "spreading" of the initial trigger population are thought to contribute to strength of the RNAi response in a number of systems and involves an RNA-dependent RNA polymerase (RDRP) (see the Perspective by **Baulcombe**). To investigate the nature of this secondary response, **Pak and Fire** (p. 241, published online 23 November) and **Sijen *et al.*** (p. 244, published online 7 December) analyzed the course of an experimentally induced RNAi reaction in the nematode worm *Caenorhabditis elegans* and also examined endogenous small RNAs. They found distinct populations of "secondary" siRNAs that are antisense to the

CREDITS (TOP TO BOTTOM): NASA PHOTOS; BORZI *ET AL.*

messenger RNA target, that have a di- or triphosphate moiety at their 5′ ends, and that may map both upstream and downstream of the original dsRNA trigger. Primary siRNAs do not appear to act as primers for RdRP, but rather guide RdRP to targeted messages for the de novo synthesis of secondary siRNAs that further boost the RNAi response.

Genome of an Often Disregarded Pathogen

Trichomonas vaginalis is a common but often neglected sexually transmitted pathogen that colonizes the urogenital tract in men and women. **Carlton *et al.*** (p. 207; see the cover) describes its genome, which at 160 megabases is significantly larger than any other parasitic protest known so far, and which provides insight into the parabasilids, which lack mitochodria and peroxisomes and instead bear organelles called hydrogenosomes. The highly repetitive nature of this genome, which expands its genome size and hence cell volume, might provide the parasite with a selective advantage for the phagocytosis of bacteria and host epithelial cells.

Separate Ways

Two dominant lineages of T cells ($\alpha\beta$ and $\gamma\delta$ T cells) are highly distinct in function and anatomical location, yet share a common precursor within the thymus. Exactly how one cell fate is decided over another remains unresolved. **Melichar *et al.*** (p. 230) present evidence that selection to the $\gamma\delta$ T cell branch in the thymus is controlled by the transcriptional factor *Sox13*, which supports and possibly even initiates $\gamma\delta$ T cell development, while opposing differentiation of their $\alpha\beta$ T cell brethren. The authors noted that SOX13 inhibited an important effector of the central T cell developmental signaling pathway mediated by the WNT protein.

Area Versus Isolation in Habitat Reduction

The worldwide expansion of urban and agricultural land has led to widespread reduction in size and increasing isolation of natural habitat patches. **Ferraz *et al.*** (p. 238) examined this phenomenon from a large-scale experimental perspective by quantifying the effects of patch size and patch isolation on the occupancy dynamics of 55 species of forest birds from the central Amazon, Brazil. Patch-size reduction had a consistently strong and negative effect on species occurrence, whereas the effects of isolation were often negative but varied considerably across species. Thus, although isolation is important, many species are absent from small patches simply because of area limitation, regardless of isolation.

One Ubiquitin, Two Ubiquitin, Three Ubiquitin, Four

The role of protein ubiquitination is well known in promoting regulated protein degradation. **Mukhopadhyay and Riezman** (p. 201) review what is known about the contribution of protein ubiquitination in other cellular pathways, including intracellular signaling, endocytosis and protein sorting.

Reconstructing Tube Worm Metabolism

The deep-sea hydrothermal vent tube worm (*Riftia pachyptila*) plays host to bacterial sulfide-oxidizing endosymbionts. These microbes have not been cultivated, inhabit a remote and nearly inaccessible environment, and form the basis for high degrees of primary productivity at deep-sea hydrothermal vents. **Markert *et al.*** (p. 247; see the Perspective by **Fisher and Girguis**) extend the metabolic reconstruction of the symbionts to reveal mechanisms of dealing with oxidative stress, two carbon fixation pathways, and the sulfide oxidation pathway. In particular, they have been able to infer relative protein stoichiometries, as well as compare symbionts in different physiological niches.

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Alan I. Leshner is chief executive officer of AAAS and executive publisher of *Science*.

Outreach Training Needed

SCANNING THE RELATIONSHIP BETWEEN SCIENCE AND SOCIETY RECALLS CHARLES DICKENS' lead for *A Tale of Two Cities*: "It was the best of times, it was the worst of times . . ." Scientific advances are coming at an unprecedented pace, and they hold great promise for further improving the human condition. The public is clearly happy about this. At the same time, however, society is exhibiting increased disaffection, fostered by instances of scientific fraud and by scientists charged with financial conflicts of interest. Perhaps worse, public skepticism and concern are increasingly directed at scientific issues that appear to conflict with core human values and religious beliefs or that pose conflicts with political or economic expediency. These include embryonic stem cell research, the teaching of evolution in schools, evidence for global climate change, and controversies over genetically modified foods. The ensuing tension threatens to compromise the ability of the scientific enterprise to serve its broad societal mission and may weaken societal support for science.

There is a growing consensus that to lessen this tension, scientists must engage more fully with the public about scientific issues and the concerns that society has about them. Efforts that focus simply on increasing public understanding of science are not enough, because the problem is not merely a lack of scientific comprehension. In some cases, the public generally does understand scientific content in a fundamental way but still doesn't like it.

Thus, the notion of public engagement goes beyond public education. We must have a genuine dialogue with our fellow citizens about how we can approach their concerns and what specific scientific findings mean. This kind of outreach is being encouraged by government agencies and private sources in Europe, Canada, and the United States. Effective public engagement requires long-term commitment, because many issues are complex and tension is persistent. The creationism/evolution issue showed us this. It would be convenient to leave this task in the hands of a few representatives selected especially for their communication skills, but that won't work. Given the breadth of issues and the intensity of the effort required, we need as many ambassadors as we can muster.

Engaging the public effectively is an acquired skill, and preparation for outreach strategies has seldom been part of scientific training programs. There are a few exceptions, including the Aldo Leopold Leadership Program and Research!America's Paul G. Rogers Society for Global Health Research. Many young colleagues are enthusiastic about discussing their work with the public, but they also are under tremendous pressure to stick to the bench, secure hard-to-get research grants, and publish rapidly and repeatedly in high-quality journals. Many even feel that the culture of science actively discourages them from becoming involved in public outreach, because it would somehow be bad for their careers.

What can be done? First, the scientific reward system needs to support our colleagues' efforts to interact with the general public concerning their work and its implications. Funding agencies such as the Wellcome Trust and the U.S. National Science Foundation and National Institutes of Health have begun encouraging the scientists they support to include outreach efforts in their proposals. Academic institutions need to join in this chorus by rewarding faculty members who fulfill commitments to such work. That will entail putting public outreach efforts among the metrics used to decide promotion and tenure.

Second, university science departments should design specific programs to train graduate students and postdoctoral fellows in public communication. Unfortunately, this means adding yet another element to already overtaxed research training programs. Many students acquire teaching experience through assistantships, but public engagement activities are different and require other strategies. We need to add media and communications training to the scientific training agenda.

This will doubtless be an additional burden on existing systems. Unfortunately, there is no alternative. If science is going to fully serve its societal mission in the future, we need to both encourage and equip the next generation of scientists to effectively engage with the broader society in which we work and live.

– Alan I. Leshner

10.1126/science.1138712

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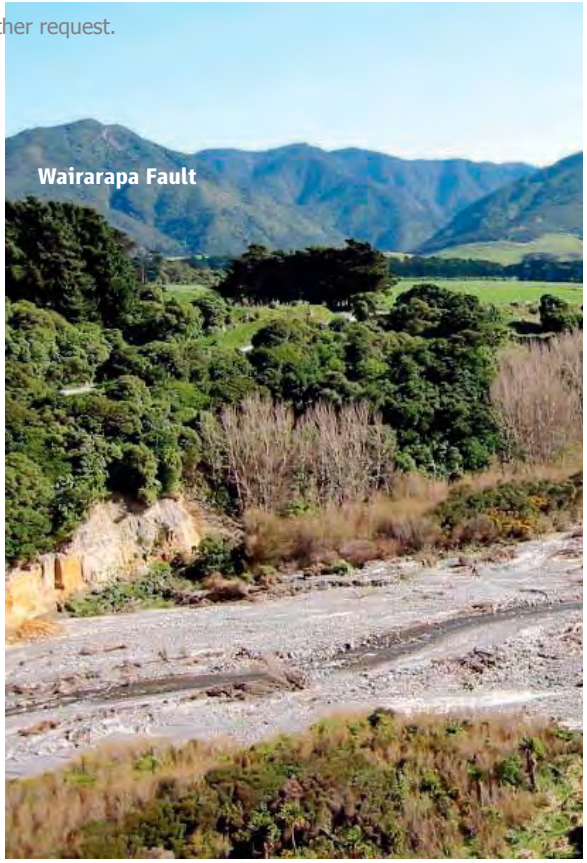
EDITED BY GILBERT CHIN AND JAKE YESTON



GEOLOGY

Upheaval Down Under

New Zealand sits astride a transition from a west-dipping subduction zone toward the north (responsible for the volcanism of the North Island) to an east-dipping subduction zone toward the south. The transition forms a system of right-lateral strike-slip faults that have produced the dramatic topography of the South Island, as well as several large earthquakes. One of these was the 1855 magnitude 8.2 temblor on the Wairarapa Fault just east of the city of Wellington. Rodgers and Little remeasured offsets produced by this earthquake and conclude that the ground slipped by as much as 18 m, an enormous amount for a strike-slip fault. For comparison, the devastating 1906 San Francisco earthquake produced a maximum of about 6 m of slip at the surface. Furthermore, the earthquake extended laterally only about 150 km (versus 480 km for the 1906 quake). An earlier quake may have produced surface slip of 14 m. The authors explain the paradox of a huge slip and short surface rupture by suggesting that the Wairarapa Fault extends deep into the crust, connecting with the northern-dipping subduction zone at depth. — BH

J. Geophys. Res. **111**, B12408 (2006).

MATERIALS SCIENCE

Adapting to the Blow

Designing equipment to protect an individual from a collision or impact often requires compromises between safety and comfort. For example, seat cushions, armrests, or headrests need to be fairly soft and compliant to be comfortable, but under these conditions they fail to absorb much energy in a collision. Deshmukh and McKinley have designed a series of adaptive energy-absorbing materials using polyurethane foams impregnated with a magnetorheological fluid (MRF). An MRF consists of a suspension of micometer-sized magnetizable particles, which flow like water under normal conditions. When subjected to a magnetic field, however, the particles align with the field to form columns or aggregates that must be deformed or broken under flow; thus the field confers considerable stiffness. This adaptability is in turn transferred to the foam when an MRF coats the struts of its open cells, offering a means of stiffening upon stress. Application of magnetic fields in the 0-to-0.2 tesla range effectively modulated the energy absorbed by these composite foams by up to a factor of 50. A scaling model allowed the authors to express all of the response data on a single curve governed by only three parameters, a convenient framework for tuning the properties of the composite. Furthermore, they envision making similar composites using a shear-thickening fluid, which responds in accordance with its rate of deformation and so would not require a magnetic field to adaptively alter its properties. — MSL

Smart Mater. Struct. **16**, 106 (2007).

MICROBIOLOGY

A Fluke Migration

Parasites in the trematode family, which includes liver flukes and schistosomes, have fantastically complicated life cycles that often involve snails and other aquatic hosts, as well as birds and mammals that prey on the intermediate hosts. Mud snails are small estuarine species that can harbor the intermediate stages of many species of trematode. A century ago on the coast of California, the Japanese mud snail was accidentally introduced when oysters were imported; it can outcompete the native snails partly because it is victimized by fewer trematodes parasites—only three.

Miura *et al.* have studied the population genetics of these traveling trematodes and have found a different itinerary for each. The most common North American species is also the most common one in northeastern Japan, whereas the rarest one was found only at Elkhorn Slough and at the original oyster source in Matsushima Bay. The third showed a striking level of genetic diversity, rarely seen in introduced species and probably due to its repeated reimportation by migrating shore birds. Before the accidental entry of its preferred host (native mud snails simply won't do), this trematode was merely a passenger in transit. — CA

Proc. Natl. Acad. Sci. U.S.A. **103**, 19818 (2006).

A free-swimming trematode.

CHEMISTRY

Pinned Propeller

Many coordination complexes have been prepared with threefold symmetry. However, exploiting chirality in such compounds tends to be challenging, in some cases because labile ligands scramble their orientation about the metal center, and in others because there is no feasible means of asymmetric induction in the synthesis, which therefore affords a racemic product mixture that must be laboriously resolved. Most chiral catalysts instead rely on a twofold symmetric motif.

Axe *et al.* have used an embedded ligand stereocenter to direct and enforce the threefold helical chirality of a tris(phenolate) titanium complex. Their tetradentate ligand consists of a central nitrogen atom bound through benzylic carbons to three alkyl-substituted phenol rings. One of these benzylic carbons also bears a methyl group in an enantiopure configuration. When the ligand reacts with a Ti(IV) precursor,

CREDITS (TOP TO BOTTOM): JOHN N. LOUIE/UNIVERSITY OF NEVADA, RENO; RYAN HECHINGER

the phenyl rings fan out in a propeller arrangement around the metal, and the methyl group induces a specific helical cant. Nuclear magnetic resonance spectroscopy confirmed exclusive formation of a single diastereomer in solution. The structure was likewise characterized in the solid state by x-ray crystallography. — JSY

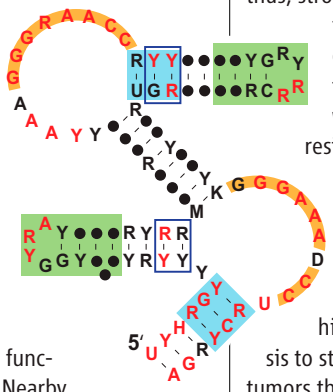
Org. Lett. 10.1021/ol062655w (2006).

MOLECULAR BIOLOGY

A Microbial Mystery

One consequence of the widespread access to DNA sequencing machines and the accompanying proliferation of genomes and genes is a renewed focus on RNA. As the most labile and hard-to-handle biological polymer, it had been valued primarily as a carrier (messenger RNA and transfer RNA) of information or as a structural, and occasionally functional, macromolecule (ribosomal RNA). Noncoding RNAs of the micro and small interfering kinds have taken center stage recently, along with riboswitches, which exhibit small molecule–induced conformational changes that regulate gene expression.

Puerta-Fernandez *et al.* have identified a large noncoding RNA (approximately 600 nucleotides in length) on the basis of a sequence that is highly conserved across 15 microbes that inhabit harsh environments (defined as extremes of pH, salt, or temperature). A consensus model of secondary structure reveals conserved regions within loops and bulges, suggesting that there are likely to be functional



A region of conserved structure.

constraints on its tertiary structure, though what this function might be is a mystery. Nearby genes do not fall into a single type of metabolic pathway, but immediately downstream in 14 out of the 15 bacterial genomes is a putative membrane protein that may form a complex with this enigmatic RNA. — GJC

Proc. Natl. Acad. Sci. U.S.A. **103**, 19490 (2006).

CELL BIOLOGY

Growing Old and Falling to Pieces

Mitochondria are the powerhouses of the cell, yet experience a remarkably tumultuous life, repeatedly fusing with each other and then parting in a fissional process. This lifestyle seems to take its toll, and in aged cells, mitochondria are often found in pieces.

Scheckhuber *et al.* examined mitochondrial morphology in yeast and in cells of a filamentous fungus as they aged. Young cells flaunted a filamentous network of mitochondria, whereas discrete mitochondria populated older cells. When mitochondrial fission was blocked, by deleting the mitochondrial fission protein dynamin-related protein 1, cells stayed younger longer and did not suffer the loss of fitness when compared to normally aging cells in other long-lived strains. It seems that reductions in mitochondrial fission can actually extend the life span of a cell, possibly by diminishing its susceptibility to mitochondria-induced apoptosis. Because similar mitochondrial dynamics are observed elsewhere, it will be interesting to see if reductions in mitochondrial fission can increase the healthy life span of other organisms. — SMH

Nat. Cell Biol. 10.1038/ncb1524 (2006).

BIOMEDICINE

Unstable Neighbors

Solid tumors may be conceptualized as a malignant mass of epithelial cells, but in fact they contain normal cells such as fibroblasts and the endothelial and smooth muscle cells that compose tumor blood vessels. The molecular conversations between malignant cells and these stromal cells can profoundly influence tumor growth; thus, stromal cells have become possible targets for cancer therapy. In contrast to tumor cells, stromal cells are widely believed to be genetically stable and hence would not be expected to develop resistance to therapy.

Pelham *et al.* have investigated the possibility that tumor-associated stromal cells, like their malignant neighbors, acquire genetic alterations during tumor progression. They used high-resolution DNA copy-number analysis to study human breast and colorectal tumors that had grown in mice for 30 to 150 days, an experimental design that allowed the stromal components to be readily identified by virtue of their mouse origin. Surprisingly, the stromal cells had undergone amplification or deletion of several genes, some of which can plausibly be linked to tumorigenesis. The magnitude of the genetic changes suggests that clones of mutant host cells had been selected for during tumor establishment or progression. Whether these changes reflect a selective pressure placed on stromal cells by the tumor in order to invoke a favorable microenvironment or, conversely, a host-initiated selection of mutant stromal cells designed to suppress tumor progression is not yet clear. — PAK

Proc. Natl. Acad. Sci. U.S.A. **103**, 19848 (2006).

CREDIT: PUERTA-FERNANDEZ *ET AL.*, *PROC. NATL. ACAD. SCI. U.S.A.* **103**, 19490 (2006)

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Pictured from left are Jeffrey Bode, Daniel Hill (Committee Chair), Melanie Sanford and Stephen Buchwald.

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University of California, Santa Barbara

Professor Melanie Sanford
University of Michigan

Distinguished Lecturer:

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Massachusetts Institute of Technology

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CONNECTED IN GINZA

Japan is ratcheting up the information age another notch—using Tokyo's famous Ginza shopping district as the test bed for a scheme to beam location-specific directions to pedestrians. Those equipped with Internet-accessible mobile phones or special hand-held terminals provided by project organizers will be able to summmon up directions or information about surrounding shops and restaurants.

The Ginza trial, to run for 3 months beginning 21 January, is part of the Tokyo Ubiquitous Network Project. The project is the brainchild of University of Tokyo computer scientist Ken Sakamura, who has made a name for himself urging that computing capabilities be built into virtually everything.

Pedestrians with hand-held terminals will have their location automatically pinpointed by some 10,000 wireless and infrared beacons and radio frequency identification tags mounted on streetlamps and buildings along roughly 12 blocks of two major streets in Ginza. They will be able to choose from options presented on the screen. People with camera-equipped phones can take a snapshot of two-dimensional bar codes placed throughout the area. They will then be connected to special Internet pages that describe what's around them and include ads for local establishments—in Japanese, English, Chinese, or Korean.

"This is at the experimental phase, but we're hoping it will be adopted widely," says Chika Satou of Tokyo's Bureau of Urban Development. She says shoppers will be surveyed for their opinions.

CREDITS (TOP TO BOTTOM): AP; ISTITUTO DI ISTRUZIONE SECONDARIA SUPERIORE; MARGE GIBSON

Creationism at the Grand Canyon

A government watchdog group is still fretting about the fact that there's a creationist book in the Grand Canyon's bookstore.

Three years ago, seven scientific groups wrote the National Park Service (NPS) asking that the bookstore remove *The Grand Canyon: A Different View*, by Tom Vail, which claims the canyon was formed about 4500 years ago, from its science bookshelf (*Science*, 16 January 2004, p. 308). In response, NPS geologists reviewed the book and concluded that it should not be sold at all. NPS officials compromised, moving the book to the store's "inspirational" section.

The Washington, D.C.–based Public Employees for Environmental Responsibility (PEER) maintains that this still violates NPS policies that all materials available to the public "should be of the highest accuracy and have undergone peer review," says its executive director, Jeff Ruch. On 28 December 2006, PEER wrote NPS Director Mary Bomar to renew its demand that the book be banned from the store. At the same time, PEER put out a press release claiming that park personnel are not permitted to tell visitors the Grand Canyon's true age of 5 million to 6 million years.

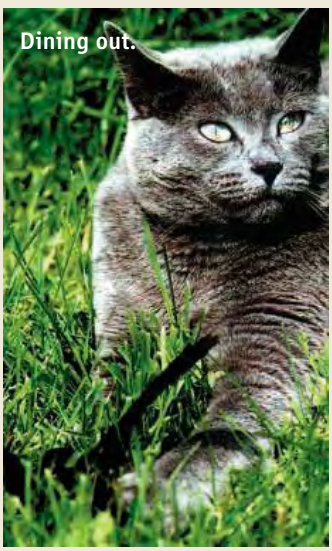
NPS has emphatically denied this charge. As for the book, Corky Mayo, NPS's manager for interpretation and education, defends the park service decision, saying, "Our job is not to convince the public how to think."

Deliciously Inefficient

Coffee may be the fuel that keeps many of us going, but a coffeepot makes a lousy engine. As part of a project to explore the physics of kitchen devices, physicist Concetto Gianino of the Institute of Advanced Secondary Instruction "Q. Cataudella" in Scicli, Italy, and his students analyzed the classic moka coffeepot—a two-chambered device that sits atop a burner. When water in the lower chamber boils, the pressurized vapor drives the remaining liquid through a filter packed with coffee and into the upper chamber. Comparing the work done pushing the water into the upper chamber to the heat energy absorbed by the boiler, the group found that the pot turned heat into work with an efficiency of 0.02%—compared to about 20% for a typical steam engine. Gianino, who reports the work in the January *American Journal of Physics*, notes in the moka's defense that its job is not to move water efficiently but to flavor it.

"This is the best way to show physics to young people," says physicist Antonino Foti of the University of Catania. "You couple the image of a coffeepot to the physics of a heat engine, and students never forget it."

Keeping Tabs on Killer Tabbies



Dining out.

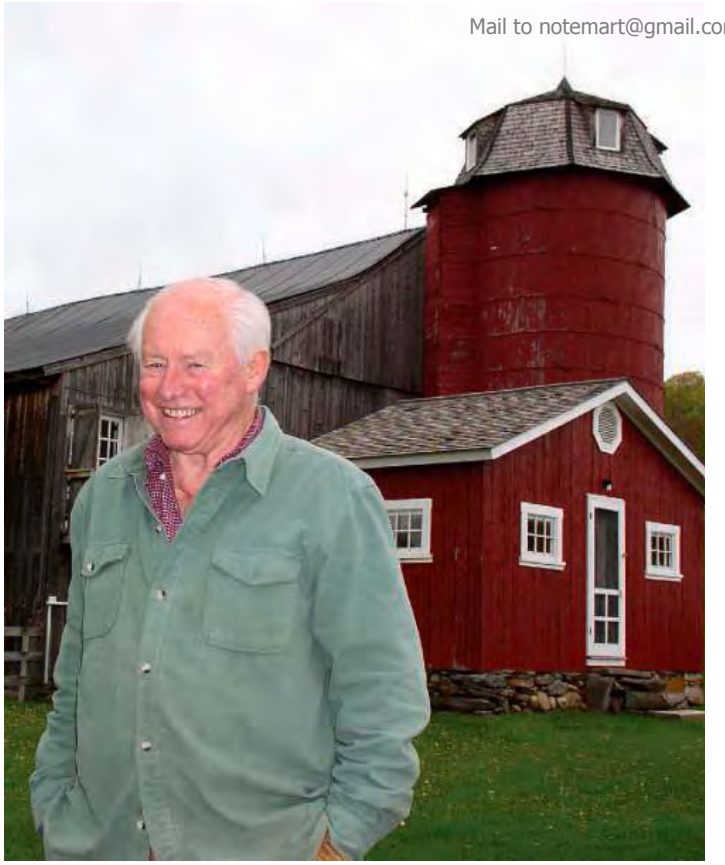
NET WATCH

They may look winsome curled up on the couch, but cats are serial killers. The estimated 90 million domestic cats in the United States slaughter more than 1 billion birds and other small animals each year. A new questionnaire from the American Bird Conservancy (ABC) in Washington, D.C., lets the general public detail attacks on wildlife by cats and other predators such as dogs and hawks.

These eyewitness reports will allow researchers at ABC to answer questions such as whether feral or pet cats take a larger toll. By comparing the results to those of previous surveys, scientists will also be able to assess whether the rising popularity of feline pets is translating into a higher body count. (The conservancy not surprisingly wants people to keep their cats indoors.)

You can read more about the impact of cats on wildlife and fill out the survey at www.abcbirds.org/cats.

EDITED BY KELLI WHITLOCK BURTON



In the News

DREAM COME TRUE. A Harvard psychiatrist celebrated New Year's Day by opening a sleep museum he hopes will awaken adolescents to the wonders of the brain.

The Dreamstage Sleep and Brain Science Museum, created by Allan Hobson, is housed in a renovated 150-year-old barn in Burke, Vermont, and features a sleep lab and videos displaying various aspects of sleeping and dreaming. "What I'm doing up here is about the brain; most people don't even know they have one," says Hobson, 73. That is, they don't understand that everything they experience "is a function of brain activity."

Hobson, former director of the Laboratory of Neurophysiology at the Massachusetts Mental Health Center, moved his equipment to Vermont in 2003 when funding cuts closed his sleep lab. A longtime advocate of education reform in medical schools and elsewhere, Hobson believes that exposing young people to the complex and fragile organ in their skulls will make them think twice before poisoning it with drugs. He plans to open the museum to educators interested in teaching students about brain function.

ON CAMPUS

SCARY DATA PROCESSING. Anne Jefferson hadn't planned on her experimental stream-flow data returning to her lab at Oregon State University in Corvallis in a police evidence bag, but it sure beat the alternative. She thought they had been blown to smithereens by police who believed the data recorders, left in the trunk of a rental car, were bombs.

Jefferson's salvation was the nature of the experiment. The small, perforated plastic tubes filled with gravel and disks with flashing green lights naturally caught the attention of an Avis car cleaner near the Minneapolis airport on 17 December. The recorders were designed to record temperature as water flows through the gravel, part of a study of gravel bed formation and evolution. Fortunately for Jefferson, the police used high-pressure water to detonate the suspected bombs, and the recorders—designed for just such a situation—continued recording.

Jefferson says she learned as much from the experience as from the data she recovered. "If you've got an opportunity to download data before you travel, do it." And of course, "don't leave things in the trunk."

MOVERS

REVOLVING DOOR. Alcino Silva was barely installed as scientific director at the Bethesda, Maryland, National Institute of Mental Health (NIMH) before he stepped down in December, just 3 weeks after giving his inaugural talk to the faculty. Silva and his boss, NIMH chief Thomas Insel, say the decision was mutual.

Silva came to NIMH in October from the faculty of the University of California, Los Angeles, with the goal of creating small, intramural labs to scout for new research projects. Insel agreed with this goal, says Silva, "but we disagreed on some details" of how to go about it. For his part, Insel says it would be "too strong" to say that he and Silva disagreed. Silva's agenda for rapid change worried senior staff; it was "not a good fit," says Insel.

NIMH Deputy Director Richard Nakamura has stepped in as acting science director. An independent review of the affair is under way to help the agency understand what went wrong. NIMH also plans a full-scale review of its research program in 2007.

Awards >>

KNIGHTED. Her Majesty the Queen rarely bestows knighthood on academics outside the United Kingdom. So the announcement that J. Fraser Stoddart, a chemist at the University of California, Los Angeles, would receive the honor was "a bolt out of the blue," he says.

Stoddart, however, is a special case. The Scottish-born researcher's work in mechanical bonds—the use of interconnected rings or ring-and-dumbbell structures—has revolutionized biochemistry, offering nanotechnology researchers a new set of building blocks not found in nature. The work could lead to advances such as molecular switches and cancer-cell detection devices.

Stoddart says some 300 former and present graduate students and postdocs had a hand in his success, as well as his late wife, Norma Stoddart, who died in 2004. "She asked the searching questions," he says.

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Getting the most out of platinum 172

Ethnicity and gene expression 173

STEM CELLS

Versatile Stem Cells Without The Ethical Baggage?

Scientists this week reported that they have isolated a new type of cell from amniotic fluid that has many of the characteristics of embryonic stem (ES) cells without the ethical baggage. But other researchers, although enthusiastic about the work, are questioning just how new these so-called amniotic fluid–derived stem (AFS) cells are and are warning that they don't eliminate the need for ES cells.

The report, published online 7 January in *Nature Biotechnology*, seems likely to throw a new twist into this week's congressional debate over legislation to expand ES cell lines available to federally funded researchers. Congressional leaders were planning to make a splash by getting both houses to pass once again a measure that was vetoed last year by President George W. Bush. But if this much-touted paper persuades the public there's a ready alternative to ES cells, "the bill won't have the impact it would have had," says bioethicist William Hurlbut of Stanford University in Palo Alto, California. The researchers themselves, led by Anthony Atala of Wake Forest University School of Medicine in Winston-Salem, North Carolina, say that AFS cells, obtained from amniocentesis samples, are no substitute for ES cells. But they see them as a unique type occupying an "intermediate" stage between embryonic and adult stem cells in terms of their versatility.

Several groups have already cultivated specialized tissue types from amniotic stem cells. But Atala insists that AFS cells are "absolutely totally different." He says they are the only amniotic cells that are "fully undifferentiated" and pluripotent—by which he means capable of giving rise to representatives of all three embryonic germ layers. He concedes, however, that it is still unclear whether AFS cells can give rise to all cell types in the body, as can ES cells.

The team, which includes researchers from Children's Hospital and Harvard Medical School in Boston, has spent the past 7 years working up their evidence that AFS cells are capable of developing into fat, bone, muscle, nerves, liver, and the lining of blood vessels. They injected human AFS cells that had been coaxed to become neural precursor cells into the brains of newborn mice and found that they dispersed throughout the brains. And cells cultivated in a bone-growing medium not only produced mineralized calcium and other bone markers but also led to the growth of chunks of bonelike material when cultured on scaffolds and implanted into mice. AFS-derived liver cells secreted urea, a liver-specific function, in test tubes. Atala said at a press conference that the group has unpublished evidence that the AFS cells can also form blood cells. It has yet to produce pancreatic beta cells, needed to treat diabetes, but Atala says, "so far, we've been successful with every cell type we've attempted."

Infant repair kit. Stem cells from amniotic fluid can be coaxed to become many different tissues. *Inset*: A 12-week-old fetus.

Like ES cells, said Atala, the amniotic cells grow rapidly, doubling every 36 hours, and the cell lines are capable of extensive self-renewal without differentiation. Unlike ES cells, they can be readily obtained from amniocentesis without harm to the donor or fetus. And they multiply indefinitely without forming tumors—a big peril with ES cells.

Atala, whose university has applied for a patent on the cell type and the team's method for isolating them, said that amniotic cells may eventually be used as a repair kit for birth defects. He also predicted that banks of cell lines obtained from 100,000 pregnancies could offer reasonably good tissue matches to 99% of the population. Some scientists are deeply impressed. "I believe … that Dr. Atala's group has discovered a new stem cell," says adult stem cell researcher Henry E. Young of Mercer University School of Medicine in Macon, Georgia.

Atala says AFS cells are the only type distinguished by C-Kit, a germ cell marker not reported in other papers about amniotic stem cells. Nonetheless, Dario Fauza of Children's Hospital, a pediatric surgeon unconnected with the Atala team who has pioneered in cultivating tissues from amniotic stem cells, says he doubts "whether they have indeed discovered a new stem cell. … I have the distinct impression we're just giving different names to the same cell." Ming-Song Tsai, a stem cell researcher at Cathay General Hospital in Taipei, Taiwan, agrees. Atala's study is "excellent," he says. But judging by surface markers and other characteristics, he believes "the cells described in this paper are the same cells" he and colleagues described last year in *Biology of Reproduction*. In that paper, the scientists reported cultivating "mesenchymal" stem cells from a single amniotic cell that could develop not only into multiple mesenchymal lineages but also into neuron-like cells. Tsai, who already has a U.S. patent on his method, adds that recently they revealed potential as liver cells.

Tsai predicts that amniotic stem cells may become a valuable tool given their "easy access [and] cultivation" and absence of ethical difficulties. But some researchers are taking a wait-and-see attitude. Harvard stem cell researcher Kevin Eggan is skeptical, especially because the field has been "burned" in recent years by hints of pluripotency in other cell types that haven't panned out.

–CONSTANCE HOLDEN

CREDITS: WAKE FOREST UNIVERSITY; (INSET) ASHLEY BRADFORD/WAKE FOREST UNIVERSITY SCHOOL OF MEDICINE

ENERGY

Consortium Wins Big Drilling Technology Contract

A consortium of big energy firms and universities has received $375 million, to be spread over 10 years, from the U.S. government for research on new ways to find and extract oil and gas. The money, awarded by the Department of Energy, comes from a controversial fund created by Congress in 2005 to encourage companies to pursue high-risk projects with potentially large payoffs. Opponents say the program is an unnecessary corporate subsidy in an era of rising energy prices.

Last week's contract awarded to the Research Partnership to Secure Energy for America (RPSEA), a Sugarland, Texas, nonprofit whose members include energy giant Schlumberger and the Massachusetts Institute of Technology, will support development of new techniques to find fossil fuels from the deepest portion of the oceans and hard-to-obtain stores on land such as tar sands. It will also fund small energy companies that have traditionally eschewed research. RPSEA's Robert Siegfried says an "academic-industry powwow" will drive research priorities.

RPSEA officials have yet to finalize the solicitation, but geophysicist Bob Hardage of the University of Texas, Austin, is hoping that the consortium will bolster his work on advanced seismic techniques for finding gas or oil in rock. Large amounts of natural gas are locked in icy cages called methane hydrates, he says, but oil firms have been leery of investing in what remains an unproven resource. "Hydrates may have great potential, or they may fall flat on their face. We need to get the answer," says Hardage. A grant that ended in 2004 from RPSEA through a federal pot of money that has since dried up attracted corporate interest in his lab's algorithms for obtaining and interpreting seismic data. The new RPSEA funding won't focus on hydrates per se but could fund basic seismic work to unlock their potential.

Floor it. New research funding could support submersible studies on deep-sea currents or geology.

Critics question why the program, created by the 2005 energy bill, is run by a third party rather than by federal officials, who would be free of corporate ties. In 2005, President George W. Bush, a former oilman, said the government shouldn't pony up even a cent because the price of oil is sufficiently high for companies to afford risky research. Last summer, the U.S. House rejected a move to cancel the program by a vote of 161 to 255.

"To call a federal R&D program a subsidy is like calling public education a social giveaway," said one supporter, Representative Ralph Hall (R–TX), at the time of the vote. RPSEA officials point to rigorous reporting and oversight requirements designed to prevent conflicts of interest. The 2005 bill also provided $125 million to be administered by the National Energy Technology Laboratory, headquartered in Pittsburgh, Pennsylvania, to fund related research. **–ELI KINTISCH**

U.S. NATIONAL SECURITY

Head of Weapons Program Fired

The U.S. government's top nuclear weapons official has been fired because of a series of security breaches at Los Alamos National Laboratory (LANL) in New Mexico. Although some legislators had previously called for the resignation of Linton Brooks, head of the National Nuclear Security Agency (NNSA), last week's announcement by Energy Secretary Samuel Bodman came as a surprise to many observers.

Bye-bye. Brooks led NNSA for 4 years.

"I've never seen anything like it," says Peter Stockton of the nonprofit Project on Government Oversight (POGO), which publicized an incident last October in which police found computer drives containing classified information from LANL during a neighborhood drug raid. "This is not a decision that I would have preferred, but it was made by a thoughtful and honorable man and is based on the principle of accountability," says Brooks, a former arms-control negotiator.

The raid followed a breach earlier in 2006 of an unclassified federal computer system, in which a hacker obtained access to information on some 1500 government employees, many with top security clearances. Brooks, who had headed NNSA since 2003, didn't notify his boss for 9 months. "I do not believe that progress in correcting these [security] issues has been adequate," Bodman said last week before naming former Brooks deputy Thomas D'Agostino as interim replacement.

Parts of the lab were shut down for as long as 7 months in 2004 after incidents involving missing disks and a laser accident. Prodded by Congress, the Department of Energy selected a new management team for the lab, which had been run by the University of California for more than 60 years (*Science*, 6 January 2006, p. 33). Some scientists fear that the new partnership, which includes the university and several major corporations, will result in more paperwork—much of it related to security and safety—and a smaller budget for the $2.2 billion facility. But the team passed its first test last fall when fewer researchers than projected chose to retire. **–ELI KINTISCH**

CREDITS (TOP TO BOTTOM): UNIVERSITY OF TEXAS; KIMBERLY WHITE/REUTERS

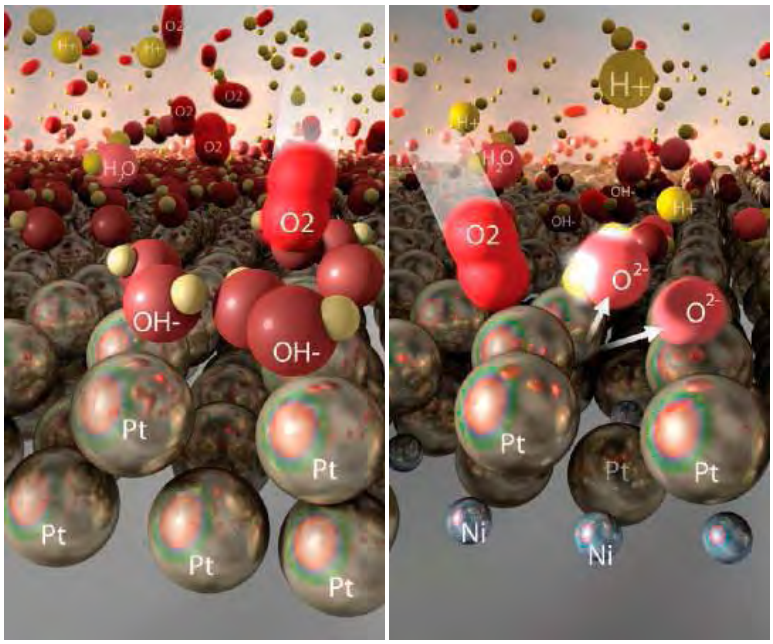
CHEMISTRY

Platinum in Fuel Cells Gets a Helping Hand

The behavior of nanoscopic bits of platinum may determine whether a hydrogen-powered car is in your future. The precious metal is the key ingredient in fuel cells that power electric cars with hydrogen, producing water as the only byproduct. Unfortunately, current models are expensive because they use so much platinum, and their performance degrades too quickly for practical use. But advances by two U.S.–led groups offer new hope for tackling these problems.

The researchers targeted what is widely considered to be the biggest concern in fuel cells: improving the performance of the platinum on the positively charged electrode, or cathode—the part of the cell where chemicals react to split oxygen molecules in half. One group, led by materials scientists Vojislav Stamenkovic and Nenad Markovic at Argonne National Laboratory in Illinois, reports in a paper published online by *Science* this week (www.sciencemag.org/cgi/content/abstract/1135941) that it increased the catalytic activity of a platinum surface 90-fold over conventional cathode catalysts used today. Meanwhile, the other group, led by chemist Radoslav Adzic of Brookhaven National Laboratory in Upton, New York, reports on page 220 that adding tiny gold clusters to the outside of their cathode materials dramatically reduced the tendency of platinum to dissolve from the cathode over extended use. "Both of these results could be quite important if the concepts can be brought to fruition in a practical manner," says Fred Wagner, a platinum catalyst expert at General Motors' fuel cell research center in Honeoye Falls, New York.

Platinum is the key to fuel cells because of its unusually high catalytic properties. This ability comes into play first at the negative electrode, or anode, to split hydrogen molecules (H_2) into two protons ($2\ H^+$) and two electrons ($2e^-$). The electrons then pass through a wire and power the car. At the end of their journey, they wind up at the cathode and pass to oxygen molecules, breaking them into negatively charged oxygen atoms (O_2^{2-}). These oxygens then pair up with protons from the anode to create water molecules. Typically, catalyzing the reactions at each electrode are platinum nanoparticles that lightly coat a high-surface-area carbon skeleton.



Loose grip. All-platinum electrodes (*left*) grab hydroxides (OH) tightly, preventing oxygen (O_2) from getting access to the catalyst. Adding nickel (*right*) softens this grip, speeding the desired oxygen-splitting reaction.

In practice, however, unwanted side reactions also occur around the cathode. Some charged oxygen atoms react with protons to create hydroxide molecules (OH) and likely other oxides as well. These oxides have an affinity for platinum atoms. They bind to the cathode surface, where they typically block access to as many as 45% of the platinum atoms, Markovic says. Even worse, the oxides tug on the platinum atoms and eventually pull many of them off the surface, drastically reducing the cathode's catalytic ability.

Researchers have made some progress on both problems by alloying platinum with other metals. In previous work, Stamenkovic and colleagues studied polycrystalline platinum electrodes alloyed with other metals and found that some of the crystalline portions seemed to perform better than others. They suspected that the disparity reflected different ways platinum atoms can pack on a surface—such as a squarelike arrangement versus a hexagonal arrangement.

To find out, for their current study Stamenkovic, Markovic, and colleagues created pure single crystals of platinum-nickel alloys with different atomic arrangements of their crystalline lattices. They compared the samples with single crystals of pure platinum as well as with conventional platinum-carbon fuel cell catalysts. They found that the most tightly packed arrangement of atoms, known in the materials lingo as a 111 surface, far outperformed all the others. The material wound up with a uniform layer of platinum atoms on top of a layer with 50% nickel atoms. All the layers under that had essentially a steady composition of three parts platinum to one part nickel (see diagram).

Stamenkovic says the group's theoretical work shows that the 111 arrangement lowers the electronic interaction between platinum atoms on the surface and oxides seeking to bind to them. The upshot is that far fewer oxides bind to the platinum surface, leaving those sites open to carry out O_2-splitting reactions. That setup boosts the PtNi alloy's activity 10-fold over a single-crystal platinum surface and 90-fold over the standard platinum-carbon combo. The reduced interaction also tugs less on the surface Pt atoms and therefore yanks fewer atoms off the surface.

That increase in stability was echoed by the result from Adzic's team. Adzic and colleagues deposited tiny gold nanoclusters on the top of a conventional carbon-platinum fuel cell cathode. They found that the clusters produced a similar change in the electronic behavior of the surface of the cathode that prevented platinum atoms from dissolving into the electrolyte, while leaving the overall oxygen-splitting activity of the platinum unchanged.

The key now, Wagner and others say, will be to create highly active, stable real-world catalysts. Markovic says his group is already working on creating octahedron-shaped platinum-nickel nanoparticles that theory shows should have all the desired 111 surfaces. If they work, hydrogen fuel cell–powered cars will take a major step toward widespread use.

–ROBERT F. SERVICE

CREDIT: CAMERON SLAYDEN/COSMOCYTE.COM

HUMAN GENETICS

In Asians and Whites, Gene Expression Varies by Race

Genetic variation among races, long a political hot potato, has also been a scientific puzzle. Although researchers have cataloged different frequencies of inherited DNA among racial groups, and physicians have found that some groups are disproportionately susceptible to certain diseases, it's not clear how or even whether the two are linked. Do subtle differences in DNA between races really matter, medically speaking?

Earlier this week, scientists described results from a new approach that may help answer that question: measuring gene expression levels among Caucasians and Asians. Because gene expression helps determine how a cell behaves, it can be more instructive than variations in inherited DNA. The researchers examined expression levels of more than 4000 genes in 142 banked cell lines drawn from individuals of European descent in Utah, and cohorts from Beijing and Tokyo. They found that 25% of the genes had expression patterns with statistically significant, although often small, differences depending on whether they came from a Caucasian or an Asian sample. Thirty-five genes had expression levels that differed, on average, as much as twofold. Still, "how that translates into traits of clinical interest is still a big question mark," says Neil Risch, a human geneticist at the University of California, San Francisco.

Express yourself. In a small sample of Japanese and Caucasian individuals, researchers found more than 1000 genes that behaved differently.

Although that critical bridge remains to be built, scientists say the expression patterns are intriguing. Indeed, geneticist Vivian Cheung of the University of Pennsylvania, who led the research team with her colleague Richard Spielman, was initially so taken aback by the number of genes whose expression varied that she suspected a technical glitch. "The 25% definitely shocked me," says Cheung, who also works at the Children's Hospital of Philadelphia.

But when she and her colleagues repeated the study on samples from 24 Chinese residents in Los Angeles, the results were virtually identical. All but one of the 35 genes with big variations in expression registered similar levels in the HapMap Asian samples and the Los Angeles cohort, they report online this week in *Nature Genetics*.

"This lends support to the idea that there are genetically determined characteristics that tend to be clustered in different ethnic groups," says Phyllis August, a nephrologist at Weill Medical College of Cornell University in New York City, who has studied variation between blacks and whites in a gene involved in hypertension. "To deny that is really denying a lot of very obvious biological truths."

Researchers are careful to say that although mean expression between Asians and Caucasians differed in more than 1000 genes studied, the expression difference between individuals from each group was often not impressive. "These averages are not absolutes," says Stephen Wooding, a population geneticist at the University of Texas Southwestern Medical Center in Dallas. He compares the variation in gene expression to height in men and women; although men on average are taller, plenty of individual women are taller than individual men.

To analyze expression levels, Cheung and her colleagues began with samples collected for the International HapMap Project, which aimed to catalog genetic variation to help identify disease genes. They used microarray technology to measure gene expression in several thousand genes at once and found measurable expression in 4197 genes. Then, they compared mean expression levels in the three different sets of samples.

At first, the researchers separated the Chinese and Japanese samples but then lumped them together after finding that only 27 genes registered different mean expression levels between the two. The different expression levels seemed to correspond to patterns of

CREDITS: PHOTOS.COM

Googling Galaxies

The computer whizzes at Google have agreed to help scientists sift through the mounds of data from a proposed telescope that aims to scan half the cosmos. Project leaders welcome Google's contribution to the $350 million Large Synoptic Survey Telescope (LSST) as they seek funding from the U.S. National Science Foundation (NSF) for most of the project.

Operating from a peak in northern Chile, the LSST would snap shots of every star and galaxy above once every 3 nights, enabling researchers to study the structure of the cosmos, probe the dark energy that is accelerating the expansion of the universe, and search for countless oddities. Google will help manage the 30 terabytes of data captured each night and develop algorithms to search through it, says J. Anthony Tyson, a physicist at the University of California, Davis, and director of the LSST project. Google was drawn to the project in part through personal connections, says Rob Pike, a computer scientist at Google in Mountain View, California, who worked at Bell Labs in Murray Hill, New Jersey, when Tyson worked there. Researchers hope to begin operating the telescope in 2014, assuming that NSF approves the project and begins funding it in 2009.

–ADRIAN CHO

Papering Over Their View?

Seeking to ward off what they see as unwarranted curbs on studies involving embryos that are part human and part animal, Stephen Minger of King's College London and four other British stem cell scientists launched a media offensive last week. They believe the public has misunderstood the purpose of such chimeras, which would be used only to derive stem cell lines for research. Currently, British law doesn't ban human-animal embryos. But in a position paper issued in December, the government hinted that because of "considerable public unease" with the prospect of chimeric embryos, it would propose a ban in a bill expected this spring. It also suggested it may grant exceptions.

Minger claims the Human Fertilisation and Embryology Authority (HFEA) told him informally that it would reject his pending research proposal involving chimeric embryos and another similar proposal pending the new bill. An HFEA spokesperson denies having done so; the agency was to make up its mind at a meeting after *Science* went to press this week.

–MARTIN ENSERINK

inherited variation in single-nucleotide polymorphisms (SNPs)—for example, if one DNA stretch with a particular SNP was rare in a higher percentage of Asians than Caucasians, average gene expression in the first group might be lower. It's still not clear whether the SNPs themselves might be regulating gene expression, or whether they travel together with other DNA that's the regulator.

The question now is whether and how these expression differences affect health. One gene, called *UGT2B17*, is deleted more often in Asians than Caucasians and had a mean expression level that was 22 times greater in Caucasians than Asians, the most dramatic variation seen. "That one really stuck out," says Wooding, who notes that this gene is involved in steroid metabolism and, possibly, drug metabolism as well.

Spielman agrees that genes such as *UGT2B17* and others that showed up in the list of 35 should be looked at individually to determine what the expression differences might mean. Next up for his group: examining gene expression in other ethnicities, including Africans, to see what patterns materialize. **–JENNIFER COUZIN**

NUCLEAR WASTE

With Plutonium, Even Ceramics May Slump

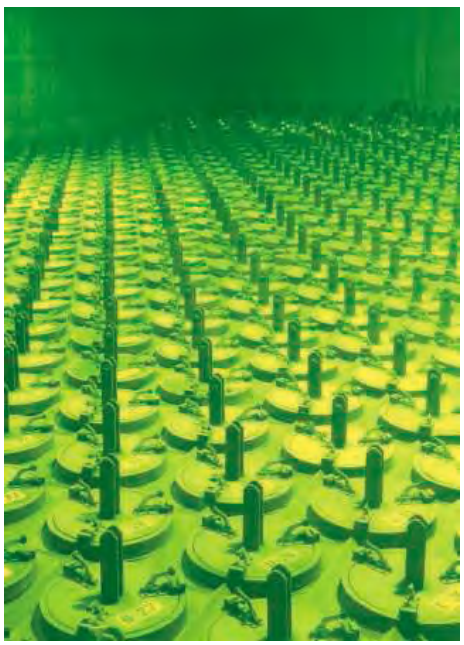
A promising idea for immobilizing nuclear waste may not be so solid after all. Researchers have pointed to crystalline ceramics such as zircon as a strong medium for holding plutonium, a fission product in spent commercial fuel and a security risk with a half-life of 24,000 years. But a new study by mineral physicist Ian Farnan of the University of Cambridge, U.K., and colleagues reveals that alpha radiation could break down this ceramic's structure more rapidly than assumed. A zircon mix containing 10% plutonium-239 (^{239}Pu), for example, could become amorphous in just 1400 years—far short of the U.S. containment target of 210,000 years. This experimental finding, experts say, points to a need for more research on alternative forms of waste storage.

Zircon ($ZrSiO_4$) is frequently studied in modeling waste storage because it can contain natural inclusions of long-lived radioactive elements such as uranium and thorium. Some such samples are as old as Earth. The Farnan study, published in the 11 January issue of *Nature*, used nuclear magnetic resonance (NMR) to directly measure the number of silicon atoms displaced by each emitted alpha particle, first in natural zircon containing ^{238}U and ^{232}Th, and then in zircon doped with ^{239}Pu. Previous estimates of such displacement were in the range of 1000 to 2000 atoms; Farnan observed a much larger displacement of about 5000 atoms, indicating that the structure would fail sooner.

Bruce Begg of the Australian Nuclear Science and Technology Organisation calls the Farnan team's work "very significant" but says it does not address the "key question": whether the alpha-induced transformation of ceramic to an amorphous state "has any detrimental impact on the ability of the waste form to lock up plutonium."

Many researchers believe it does. Linn Hobbs of the Massachusetts Institute of Technology Department of Nuclear Science and Engineering says that a form that becomes amorphous can change "the way that various elements are surrounding other elements." This could allow significant "dimensional changes" in the structure, according to Hobbs, which "may or may not have larger leach rates" into the surrounding environment.

The U.S. storage plan for a significant portion of its weapons waste relies on a completely amorphous medium: glass. The U.S. Department of Energy (DOE) is melting radioactive material together with borosilicate glass in a program to immobilize millions of liters of mixed liquid waste at the Hanford Nuclear Reservation near Richland, Washington, and the Savannah River Site near Aiken, South Carolina. DOE chose this "vitrification" option because tank waste is so complex that no single crystal structure could accommodate all its components. However, most of the plutonium and uranium has been removed, so "there's essentially no probability of a criticality event" in vitrified tank waste, says J. Russell Dyer, chief scientist with DOE's Office of Civilian Radioactive Waste Management. The U.K. and France also vitrify reprocessed power-plant fuel, but only after removing the plutonium.



In limbo. Spent reactor fuel at the Idaho National Laboratory awaits its fate.

CREDIT: PETER ESSICK/AURORA/GETTY IMAGES

The biggest reservoir of plutonium-bearing waste is in spent but unreprocessed commercial nuclear power fuel, most of it stored onsite at utility companies, expected to reach 62,000 metric tons by 2010. The federal weapons complex owns about 7000 metric tons of reprocessed weapons waste and spent fuel, also containing plutonium. Experts say that research is needed to narrow down the candidates for optimal plutonium storage.

Vitrification is a "completely unstable" method of storing wastes, says Kurt Sickafus of the Materials Science and Technology Division at Los Alamos National Laboratory in New Mexico. He argues that ceramic forms can be made "highly stable," but not the silicate-based forms such as zircon. He suggests fluorite crystal structures instead because their amorphousness lies somewhere between that of glass and the rigid silicates. This makes them able to tolerate radiation-induced defects without severe disruption of the crystal lattice, he says. Other researchers look to pyrochlores and zirconolites, outgrowths of the work on the titanium-based SYNROC ("synthetic rock") by A. E. Ringwood in the 1970s. U.S. funding for research on ceramic waste forms has been stagnant or declining for years, says Sickafus.

Despite the obstacles, Farnan says the problem is "tractable." However, "if you take a material and ask what its behavior is going to be in 10,000 years, the uncertainties become very large." Even so, there is good news in these findings, Sickafus notes: This "very sensitive and elegant" NMR technique can help whittle down uncertainty about the robustness of alternative materials relatively quickly.

–VALERIE BROWN

Valerie Brown is a writer in Portland, Oregon.

In the pipeline. Offshore fish farms are expected to boom.

CREDIT: NOAA

OCEAN POLICY

Panel Urges Environmental Controls On Offshore Aquaculture

A blue-ribbon panel is calling for tight environmental standards on farmed fish in U.S. ocean waters. Although few commercial aquaculture operations currently exist outside shallow coastal zones, the panel predicts a boom in offshore enterprises and says now is the time to craft regulations to prevent future ecological damage. Many of the recommendations in the 142-page report, released this week, would also help make existing aquaculture operations more benign and sustainable, the panel says. "I think the report makes very helpful, practical recommendations, anchored in good science," says Jane Lubchenco of Oregon State University in Corvallis, who wasn't involved.

Farmed fish and other fruits of marine aquaculture—worth $200 million in the United States last year—are currently grown within 5 kilometers of shore, a swath of water regulated by states. But heightened demand and new technologies, such as storm-resistant pens, are promising to carry fish farming into open waters under the jurisdiction of the federal government. In 2005, the Pew Charitable Trust and Lenfest Foundation asked the Woods Hole Oceanographic Institution (WHOI) in Massachusetts to convene a task force of stakeholders to examine the risks and benefits of offshore aquaculture and how it could be regulated.

First off, says panel chair Richard Pittenger, a former vice-president of WHOI, Congress should put the National Oceanic and Atmospheric Administration (NOAA) in charge and mandate it to evaluate the risks of offshore aquaculture before granting any permits. In the panel's view, major hazards include pollution from excess waste and feed. Although the open ocean is better than coastal ecosystems at dispersing these pollutants, the panel says that water-quality standards are needed. Another worry is that escaped fish could harm wild populations. That's why the panel says non-native fish, regardless of whether they're in coastal or open waters, should not be allowed, unless they have been shown to pose no risk.

In addition to strict regulations, the panel also called for market-based incentives that would encourage industry to invest in sustainable aquaculture operations. "I think we've arrived at a reasonable balance that would improve the environmental performance of the U.S. industry," says task force member Bill Dewey of the Taylor Shellfish Company in Shelton, Washington. Also important for the long-term health of aquaculture is reducing and replacing fishmeal. On average, it takes 6.6 kg of wild-caught fish to grow 1 kg of farmed fish, the panel notes, and the supply fisheries are fully or overexploited.

NOAA wants to add offshore aquaculture to its bailiwick. In the previous Congress, the agency proposed a bill—the National Offshore Aquaculture Act of 2005—that would give it authority in federal ocean waters. Opponents criticized the bill because it didn't mandate environmental standards (*Science*, 8 September 2006, p. 1363), and it never made it out of a Senate committee. Two key senators are expected to reintroduce the bill, which NOAA is revising to include recommendations from the panel. **–ERIK STOKSTAD**

SCIENCESCOPE

Korea Boosts R&D Spending

Nuclear fusion research gets a 20% increase in South Korea's new science budget, thanks to the soon-to-be-completed Korea Superconducting Tokamak Advanced Research facility and the country's involvement in the International Thermonuclear Experimental Reactor. "The Korean government decided fusion should be one of our major R&D efforts," says National Fusion R&D Center Director Kyoungsoo Lee about the spending boost, to $72 million. Overall, spending on science and technology will rise 9.6% to $10.4 billion, making 2007 the second year in a row in which research receives the largest percentage increase of any budget sector.

–DENNIS NORMILE

An Italian Welcome Mat

Italy hopes to attract more foreign scientists to its universities by offering them salaries and tenure comparable to what their Italian colleagues receive. Fabio Mussi, Italy's minister of universities and research, has set up a special government fund to finance the changes; meanwhile, support for ongoing research projects will be reviewed in hopes of finding money to stabilize current academic positions.

Scientists like the idea but are waiting to see how Mussi will follow through. Elisa Molinari, a physicist and director of the Italian Institute for the Physics of Matter, believes that a better way to attract "brilliant brains from all over the world" would be for the government to spend more on research.

–FRANCESCO DE PRETIS

Poached Eggs

An international body has ended a ban on exporting caviar, or sturgeon eggs, from the Caspian Sea. Last week, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) allowed exports of stellate and Russian sturgeon caviar to resume, noting "improvements to the monitoring programs" of Russia, Kazakhstan, Turkmenistan, Azerbaijan, and Iran.

The move comes despite evidence that sturgeon poaching is rampant. Phaedra Doukakis of the Pew Institute for Ocean Science in New York City says that CITES "gave no evidence the species were recovering. This decision flies in the face of its principle to allow trade only when it demonstrably does not jeopardize the survival of a species." CITES limited the total exports to 86 tons, 15% less than in 2005, the last year exports were permitted.

–CHRISTOPHER PALA

Agent Orange's Bitter Harvest

New findings paint a more sinister picture of the Vietnam War herbicide; scientists are trying to revive an epic study of its effects on U.S. veterans and clarify its legacy in Vietnam

HANOI—Several children and young adults sit at a table, fiddling with plastic blocks and colored rings with the self-absorption of toddlers. "We teach them small skills. How to wash hands. How to play with toys, distinguish colors," explains Nguyen Thi Oanh, a teacher at Friendship Village, a rehabilitation center in Van Canh, west of Hanoi. The students, 9 to 24 years old but with limited mental development, will spend a few years here and then return to their home villages. During rehab, Oanh says, "some kids get a little bit better." Others do not.

This scene may resonate among health workers around the world who have run similar rehab sessions. But in Vietnam, it resonates with the trauma of war. The 120 children and young adults from 34 provinces at Friendship Village share one thing in common: Their parents or grandparents claim to have been in areas where the U.S. military 4 decades ago used herbicides—the most notorious being Agent Orange—to destroy crops and strip forest canopy to flush out the enemy.

Vietnam claims that the children's disabilities were caused by parental exposures to Agent Orange. Western scientists have long been at odds with their Vietnamese counterparts over the strength of evidence correlating exposure to dioxin—a toxic contaminant of the herbicide—and illnesses in individuals, particularly birth defects. "The Vietnamese government is using malformed babies as a symbol of Agent Orange damage," says Arnold Schecter, a toxicologist at the University of Texas School of Public Health in Dallas, who remains cautious about making associations after studying Agent Orange for more than 20 years.

In Vietnam, there is far less ambiguity. "The number of child victims could be in the 100,000s," says Dang Vu Dung, director of Friendship Village, run on donations from overseas veterans. Countrywide, roughly 3 million people are Agent Orange victims, asserts Nguyen Trong Nhan, vice president of the Vietnam Association for Victims of Agent Orange/Dioxin (VAVA), a nongovernmental organization in Hanoi.

The long-term effects of Agent Orange may never be known, now that an ambitious attempt to analyze them has ended. Late last year, the U.S. Department of Defense pulled the plug on a 20-year-long health study of U.S. veterans involved in Operation Ranch Hand, which sprayed 95% of the Agent

CREDIT: AP

Sowing trouble. U.S. Air Force planes spray Agent Orange defoliant over Vietnam in 1966.

CREDIT: AP

Orange and other herbicides used in Vietnam. The $140 million research effort was "the most detailed study of human exposures ever done," says epidemiologist Joel Michalek of the University of Texas Health Science Center in San Antonio, who until 2005 was a principal investigator of the Air Force study. The firmest link it uncovered was between Agent Orange and an elevated risk of diabetes. Otherwise, Michalek says, "there has been little or nothing to say—until now." A cancer signal is just beginning to emerge from the data, he claims, as are subtle physiological changes such as suppressed testosterone levels and prostate growth.

The decision to halt Ranch Hand stunned many researchers. "It will be a tremendous loss to science if it is not continued," says Linda Birnbaum, chief of the U.S. Environmental Protection Agency's (EPA's) experimental toxicology division in Research Triangle Park, North Carolina. A proposal to resurrect it is circulating on Capitol Hill. By law, the Air Force must transfer custody of existing Ranch Hand data and specimens to the U.S. National Academies, which hopes to make them available for further research.

Another day of reckoning is on the horizon—this one for the Vietnamese who claim to have been injured by Agent Orange. This spring, in a U.S. appeals court, oral arguments are expected to begin in a class-action suit brought by Vietnamese citizens against Agent Orange manufacturers. (The claims had been dismissed by a lower court in 2005.) The claimants demand compensation like that given to U.S. veterans who handled Agent Orange and contracted certain illnesses. "It is time for the U.S. government and chemical companies involved in the war to take responsibility for the damage caused by their actions and products," says epidemiologist Tuan Nguyen of the Garvan Institute of Medical Research in Sydney, Australia.

Bitter feelings threaten the blossoming relationship between the United States and Vietnam. "Agent Orange is a very sensitive, very delicate, very political issue—and very controversial," Schecter says. In a small gesture, the U.S. government has pledged to assist Vietnam in cleaning up several hot spots where soil dioxin levels are sky-high.

Researchers from both countries hope this will kindle fresh interest in a joint probe. "We are really ready for cooperation with the United States—as long as it is based on mutual benefits and mutual respect," says toxicologist Le Ke Son, director general of Vietnam's "national steering committee for the overcoming of the consequences of toxic chemicals used by USA in the war in Vietnam," or simply "Committee 33." But U.S. experts have found Committee 33 rigid and opaque and therefore hard to work with. Says Michalek, "Studies in Vietnam are going to be difficult."

True colors

The U.S. and South Vietnamese air forces, mostly using military transport planes, began spraying herbicides in the fall of 1962. Over the next decade, they unloaded some 77 million liters of herbicides on 2.6 million hectares of south and central Vietnam. For the first few years, the main herbicide was Agent Purple, a mix of 2,4-dichlorophenoxyacetic acid (2,4-D) and two forms of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Then in 1965, the military deployed Agent Orange, a faster-acting defoliant consisting of 2,4-D and a single form (*n*-butyl ester) of 2,4,5-T. In a painstaking reanalysis of herbicide use during the Vietnam War, Columbia University chemist Jeanne Mager Stellman and her colleagues estimated that over 6 years, 45 million liters of Agent Orange were sprayed (*Nature*, 17 April 2003, p. 681).

Chemical clearance. Normal mangroves (*top*) and a forest 5 years after defoliation.

These agents were laced with a long-lived contaminant, 2,3,7,8-tetrachlorodibenzoparadioxin (TCDD). It's unclear precisely how much dioxin rained down on Vietnam. Stellman's group adopted a "conservative" value of 3 parts per million of TCDD in Agent Orange, although levels "could be fourfold or more higher," they assert. About 10% of Vietnam took a direct hit.

By the late 1960s, Western researchers had evidence that 2,4-D and 2,4,5-T cause birth defects in mice; they were alarmed as well by anecdotal reports of birth defects in Vietnam attributed to the herbicides. In a resolution at its annual meeting in 1969, AAAS (publisher of *Science*) urged the Defense Department to "immediately cease all use of 2,4-D and 2,4,5-T in Vietnam." As criticism of the war intensified, the U.S. military banned the herbicides in April 1970, although Ranch Hand operations didn't cease until late in 1971, and South Vietnamese forces continued to dip into herbicide stockpiles until the war ended in 1975.

But whereas 2,4-D and 2,4,5-T "are not innocuous compounds," Birnbaum notes, evidence soon pointed to a darker villain: dioxin.

A toxic trail

In the past 3 decades, studies have revealed that dioxin causes many harmful effects in animals—birth defects, cancers, and endocrine disorders—sometimes at vanishingly low concentrations. In a rogue's gallery of 75 known forms of dioxin, TCDD is the nastiest. "From fish through primates, it's the most toxic," Birnbaum says, perturbing "lots of different systems in the body." Significantly, it binds to the aryl hydrocarbon receptor, a key regulatory protein. As a result of this unholy coupling, dioxin throws a wrench into processes as diverse as normal homeostasis and aging. (Ukraine's president, Victor Yushchenko, was deliberately poisoned with TCDD in 2004.)

It has, however, been difficult to probe for links between dioxin and human illness. "Thank goodness, very few people in the world are ever exposed to high levels," Birnbaum says. But those with high exposures—in rare occupational accidents and industrial disasters—have suffered chloracne, a severe skin disorder, and transient symptoms of poisoning. Studies have also indicated that dioxin might trigger or abet cancer development and possibly heart disease years after exposure.

Exposures in Vietnam are hard to quantify. Stellman's team estimates that more than 3000 villages with at least 2.1 million people were "sprayed directly" with herbicides, although the number potentially exposed could be as high as 4.8 million. "There are no good records as to who lived in a certain village at a certain time," says Michalek. In more than 30 trips to Vietnam since 1983 to document TCDD in humans, wildlife, food, and

Potent symbol. Children of parents or grandparents exposed to Agent Orange attend a rehabilitation center at Friendship Village near Hanoi; Vietnam blames their problems on Agent Orange.

soil, Schecter and John Constable of Harvard University have found elevated dioxin levels in many of the roughly 4000 people they have tested. Schecter says that a handful of individuals living near a wartime herbicide storage area, Bien Hoa, had TCDD blood levels exceeding 400 parts per trillion. (The U.S. population averages 1 or 2 ppt.)

In the United States, in response to pressure from veterans' groups, the Air Force in the late 1970s began planning a study to track the health of some 1200 Ranch Hand veterans and a control group: veterans not exposed to Agent Orange. The research also examined both cohorts' roughly 8500 children. "We launched the study knowing next to nothing about the exposure profiles"—how much dioxin each vet absorbed, says Michalek, who started on the project in the late 1970s when he was with the Air Force Research Laboratory at Brooks Air Force Base in Texas.

With veterans blaming Agent Orange for an array of ills, the Air Force scientists opted for a broad approach to data collection—and took some heat for that. "The study was seen as seriously flawed," asserts Stellman, who states that it began as "too much of a fishing expedition, measuring everything and anything with too few scientific hypotheses."

In 1987, Ranch Hand researchers began to measure dioxin levels in veterans' blood samples. It was revelatory. "Many people who thought they were highly exposed actually were not," says Birnbaum. "There were very few people with high levels." Michalek and his colleagues sorted veterans into low-, medium-, or high-exposure categories. In 1995, that rough cut at estimating exposure turned up a clear hit: Diabetes risk increased with exposure. Over the next decade, however, other findings were frustratingly indistinct.

Michalek has since reanalyzed the data, zeroing in on veterans who were in Vietnam during or prior to 1968 and were involved in at least 90 days of herbicide spraying. He also excluded vets who spent more than 2 years in Southeast Asia. (Veterans in the control group with such extended deployments are at higher risk of cancer—possibly from exposure to DDT during a World Health Organization campaign in the 1960s to eliminate malaria in the region, Michalek speculates.) The new analysis uncovered "a stronger and clearer trend" of a dose-dependent risk for diabetes and cancer, says Michalek, who intends to submit his findings to a peer-reviewed journal later this month. He expects heavy flak: "Critics will accuse me of slicing and dicing the data," he says.

He and others say it would be a mistake to walk away now. "Certain chronic effects can take years and years to develop," says Birnbaum. And although some experts assailed the study's design, a panel of the National Academies' Institute of Medicine (IOM) concluded last year that "the data appear to be of high quality and the specimens well preserved." The Air Force will transfer Ranch Hand data and specimens to the academies by the end of September. "If we subsequently receive funding to manage the assets and permission from the research subjects, we intend to make the materials available for further analysis," says David Butler, an IOM senior program officer. And IOM next month will convene a panel to advise the Department of Veterans Affairs (VA) on how to apply the Stellman group's exposure model to studies of U.S. veterans. Michalek's university, meanwhile, sent a proposal late last year to several members of Congress and key committees seeking support for a $2-million-per-year Ranch Hand extension.

Congress has intervened before: It passed the Agent Orange Act in 1991, mandating care for veterans known to have been exposed to Agent Orange. The act also called for a definition of illnesses attributable to Agent Orange, as a basis for compensating sick veterans. Toward this end, the VA enlisted IOM to review the health effects of exposure to herbicides used in Vietnam. IOM's landmark report, *Veterans and Agent Orange*, came out in 1994; by law it must be updated every 2 years until 2014. The latest update, published in 2004, concludes that there is "sufficient evidence of an association" between herbicide exposure and five ailments: chronic lymphocytic leukemia, soft-tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, and chloracne (see table).

Of all categories of illness blamed on Agent Orange, the most divisive, perhaps, is birth defects. This "remains one of the most contentious issues in science," says Nguyen of the Garvan Institute. According to VAVA's Nhan, the rate of severe congenital malformations in herbicide-exposed Vietnamese populations is 2.95%, compared to 0.74% in nonexposed populations. Grandchildren are afflicted at a similar disproportionate rate, Nhan notes. Government publications about the herbicides are filled with heartrending pictures of deformed children. Reports of families with multiple malformed children abound.

In contrast, the IOM panel has noted "limited or suggestive" evidence linking herbicide exposure and one congenital defect: spina bifida, a malformation of the spinal

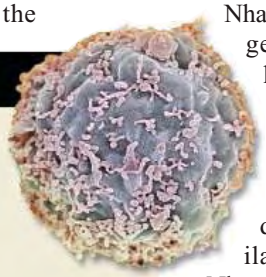
Herbicides and Ill Health

SUFFICIENT EVIDENCE OF AN ASSOCIATION

Chronic lymphocytic leukemia (*right*)
Soft-tissue sarcoma
Non-Hodgkin's lymphoma
Hodgkin's disease
Chloracne

LIMITED OR SUGGESTIVE EVIDENCE OF AN ASSOCIATION

Respiratory cancer (lung and bronchus, larynx, and trachea)
Prostate cancer
Multiple myeloma
Early-onset transient peripheral neuropathy
Porphyria cutanea tarda
Type 2 diabetes mellitis
Spina bifida in offspring of exposed individuals



CREDITS (TOP TO BOTTOM): MUTSUMI STONE; STEVE GSCHMEISSNER/PHOTO RESEARCHERS INC.; SOURCE: INSTITUTE OF MEDICINE

cord. For all other birth defects, the panel concluded that evidence for an association was "inadequate or insufficient."

This long-running debate has been reignited. A team led by Nguyen for the first time pooled published data with unpublished data from Vietnamese studies of veterans and sprayed civilians. Their meta-analysis of 22 studies, half of which were unpublished, found a "substantially greater" association between Agent Orange exposure and birth defects in Vietnamese populations than in U.S. veterans. Overall, people who believe they were exposed to Agent Orange were almost twice as likely to have a child with birth defects as were unexposed people, Nguyen's group reported last October in the *International Journal of Epidemiology*.

The study has received mixed reviews. "I don't think using unpublished data is a good way to do a meta-analysis," says Schecter, who believes that poor nutrition, infections, and genetic flaws are responsible for most malformations seen in Vietnamese children. Michalek, on the other hand, says Nguyen and colleagues "did the best they could with available data." Nguyen notes that the Vietnamese researchers have had a "hard time" submitting their findings to international journals. "I certainly hope that they will publish their work," he says.

Whether the health effects can be brought into sharper focus is unknown. A few years ago, prospects were looking good. In March 2002, the U.S. and Vietnamese governments signed a research framework to probe Agent Orange effects. "Agreeing to do the research is the easy part," Anne Sassaman, then an official with the U.S. National Institute of Environmental Health Sciences (NIEHS), said at the time. "The more difficult task will be to develop research studies that are definitive and address the underlying causes of disease in Vietnam."

NIEHS thought it had a viable project in sight. In 2003, the agency committed $3.5 million to a study led by David Carpenter of the University at Albany in New York, to probe the possible relation between Agent Orange and birth defects. But talks over a U.S.–Vietnam cooperation agreement foundered. "Without it, the research was impossible to implement," says Committee 33's Son. U.S. officials, including the ambassador to Vietnam and the health attaché, "worked very hard with the Vietnamese but ran into constant roadblocks," says one U.S. scientist. With talks stalemated, NIEHS shelved the Albany study in February 2005.

CREDIT: MUTSUMI STONE

Seeking closure

In a common room of a dormitory at Friendship Village, Tran Van Tham, a retired lieutenant in the Vietnam People's Army, and several other veterans are lounging under a portrait of Ho Chi Minh, the leader with the white-streaked Fu Manchu mustache and goatee who orchestrated the North's victory 30 years ago. Whereas disabled children stay for rehabilitation for up to 3 years, veterans cycle through for a month at a time for health checks. "We reminisce, but mostly are here to enjoy life. We feel better, spiritually," says Tham.

Years ago, Tham's two babies succumbed to hydrocephaly and other defects, he says. He blames wartime Agent Orange exposure. Nevertheless, Tham says, eyes glistening, "we can forgive American veterans." But Agent Orange victims are a burden on Vietnam, he says. "We support our government's policy to close the past and look to the future with the United States," adds Nhan. "But we cannot ignore Agent Orange victims." In 2000, Vietnam introduced a program to compensate people who claim disability from Agent Orange exposure. But Nguyen says that each person gets only a few U.S. dollars per month. He estimates that Vietnam needs hundreds of millions of dollars to care for all victims.

"We can forgive American veterans. But **Agent Orange victims are a burden on Vietnam."**

—Tran Van Tham, Vietnam People's Army

In 2004, VAVA, exasperated after years of pleas for U.S. aid went unanswered, filed a class-action suit in U.S. District Court against 37 companies that supplied herbicide chemicals to the U.S. military during the Vietnam War. "We had hoped the United States would respond with goodwill and regarded the lawsuit as a last resort," says Nhan.

The claims were dismissed in March 2005. In a 233-page decision, Senior District Judge Jack B. Weinstein ruled that the companies could not be sued as government contractors. Nor was he persuaded by the scientific case. "No study or technique presented to the court has demonstrated how it is now possible to connect the herbicides supplied by any defendant to exposure by any plaintiff to dioxin from that defendant's herbicide," he wrote. The decision "was a great surprise," says Nhan. The plaintiffs appealed to the 2nd Circuit Court of Appeals in New York City, and oral arguments could be heard as early as April.

The plaintiffs' first challenge is to convince the appeals court that the companies can be sued. If they succeed, they would then have to refute Weinstein's conclusions about the science. "The fact that diseases were experienced by some people after spraying does not suffice to prove general or specific causation," the judge wrote. "Proof of causal connection depends primarily upon substantial epidemiological and other scientific data."

That's a tough argument to overcome, given the paucity of solid epidemiological data. To carry out a high-quality study of human health effects in Vietnam would require "a huge amount of money," says Birnbaum. The "real hurdle," adds Sassaman, who recently retired from NIEHS, "is to get the appropriate scientists and scientific expertise engaged in truly collaborative research." With that in mind, she says, NIEHS has just launched a program to fund junior researchers from Vietnam and other developing countries to work up to 2 years in labs of NIEHS-funded scientists.

Others are taking direct action to eliminate dioxin hot spots in Vietnam. International experts, working with Vietnamese counterparts, have identified nearly 100,000 square meters of heavily contaminated soil in several places where herbicides were stored during the war, says Son. Near Da Nang Airport, he says, TCDD levels in soil reach 35 parts per billion—35 times the permissible level. "Hundreds of thousands of tons" of soil will have to be dug up and stored or treated to remove dioxins, Son says. Last month, the Ford Foundation awarded $460,000 to Hatfield Consultants, an environmental firm in West Vancouver, Canada, to assist at Da Nang.

The U.N. Development Programme, with support from EPA and the Ford Foundation, is setting up a $60 million trust fund for cleanup efforts and to improve the economy of villages near the hot spots. Vietnam's Ministry of Defense has already commenced cleanup at Bien Hoa. "We should get rid of these hot spots," says Birnbaum. "We know that dioxin is bad stuff."

There may be no consensus on exactly how potent dioxin is as a cause of disease and disfigurement. But people do seem to agree that purging the land of the last vestiges of the Vietnam War—particularly the chemical residues of Agent Orange—is something worth fighting for.

–RICHARD STONE

En garde! In a "fencing" move, one fruit fly (*left*) thrusts a foreleg toward an opponent.

NEUROBIOLOGY

Fruit Fly Fight Club

Fruit flies brawl over mates and territory. Now some scientists are betting that these battles can help them unravel the genetic basis of aggression

BOSTON, MASSACHUSETTS—After a furious combination of blows, the pugilist has his opponent backed up to the edge of the ring. Punches fall like rain as the opponent teeters on the brink. But just when it looks like he can take no more, the fighter employs a surprising tactic. Planting one wing on the ground, he regains his balance and drives back his adversary with four wildly swinging legs. The combatants here are fruit flies, the ring is a thimble-sized cup of agar, and the fighting venue is a laboratory here at Harvard Medical School.

Sibu Mundiyanapurath, a visiting medical student from Germany, is reviewing videos of a recent series of bouts with a genetically modified strain of *Drosophila melanogaster*. Even unaltered fruit flies fight, Mundiyanapurath says, but this strain is unusually combative. "These guys just keep on going after each other," he says.

Who knew fruit flies were such pugnacious little beasts? Very few people until recently, says Harvard neurobiologist Ed Kravitz, Mundiyanapurath's research adviser. In a research paper published in 1915, the noted geneticist Alfred Sturtevant mentioned tussles between male flies competing for mates, but only a smattering of papers on fly aggression appeared subsequently in the scientific literature. That seems to be changing now.

Since 2002, Kravitz and colleagues have described a surprisingly diverse repertoire of aggressive behaviors in these tiny insects. They've recently found that flies remember previous opponents and that vanquished flies seem to develop a "loser's mentality" that virtually ensures defeat in subsequent bouts. The biologists also discovered that male and female flies have distinct fighting styles, and they have taken advantage of the powerful genetic techniques available to *Drosophila* researchers to investigate the basis of such differences.

Other scientists have turned into fruit fly fight promoters too. Last year, researchers in California and North Carolina independently reported on changes in gene expression in fly strains bred for aggression. Understanding the genetic basis of aggression in flies may eventually lead to a better understanding of aggression in other animals, including humans, Kravitz and others suggest. "*Drosophila* are a great model system for looking at the genetic basis and evolution of aggressive behavior," says Ary Hoffmann, a geneticist at the University of Melbourne in Australia who published a series of papers on fly aggression in the 1980s. Hoffmann had shelved his work on aggression, but he says the new research has rekindled his interest, and his lab now plans to look for genetic variations that account for individual differences in fly aggression.

Lobster versus fly

Kravitz first heard of fighting flies about 10 years ago when he gave a lecture on his studies of aggression in lobsters. That research, begun in the late 1970s, had established that levels of neurotransmitters such as serotonin fluctuate when the crustaceans fight to establish social status. Afterward, someone from the audience told him about fighting flies. "I don't think I was too impressed," Kravitz recalls. But the researcher sent him some papers, and that got Kravitz thinking about the advantages of working with the insects.

One of the most fundamental questions he'd been trying to address was how complex patterns of behavior get wired into nervous systems. "If you want to ask a question like that, you have to be able to manipulate genes," Kravitz says. "And there was no easy way to do that with lobsters."

Before he started tinkering with fly genes, Kravitz wanted a better understanding of the insect's fighting behavior. Many of the early experiments put a bunch of flies, males as well as females, in a small space. It was basically a free-for-all, with courting, fighting, and mating going on simultaneously. Kravitz simplified the situation by pitting just one fly against another. It took some trial and error to get the setup right, but the arena now consists of a small cup of agar enclosed by Plexiglas. A dab of yeast paste—a delicacy for *Drosophila*—in the middle of the cup gives the flies something to fight over. For male flies, the researchers up the ante by sticking a headless female in the center of the ring. (The males seem to find decapitated females just as attractive as intact ones, and the headless ones can't fly away.)

After poring over more than 2000 videotaped interactions between male flies, Kravitz and colleagues identified nine distinct acts of aggression in a 2002 paper in the *Proceedings of the National Academy of Sciences* (*PNAS*). These moves included "wing threats" in which one fly faces another and suddenly raises both wings, "fencing" in which one fly pokes a leg at another fly, "lunges" in which one fly stands up on two hind legs and slams down on his opponent, and "boxing," which looks about like it does in humans, if you add two limbs and subtract the gloves.

Whichever fly started the fight was most likely to win, especially if his first move was a strong one, the researchers also found. For example, an instigator that used a slow "approach" move, in which he lowered his body and walked toward his opponent, had 3-to-1 odds of ultimately making his opponent retreat. But flies that started with a more intense move, such as fencing or wing threat, improved their odds to 16 to 1.

"The videos were just absolutely stunning," says Robert Huber, an animal behaviorist at Bowling Green State University in Ohio who helped Kravitz with some of the behavioral analysis. What struck Huber most about the fly fights were the intricacy of the different moves and the fact that the insects used certain combinations far more often than

CREDIT: STEVEN P. NILSEN

others. "It all seemed to be going on according to very strict rules," he says. Huber speculates that a consistent pattern of fight escalation gives the insects an efficient way to establish dominance hierarchies: Fights between mismatched flies get resolved quickly with visual displays and other low-intensity maneuvers, whereas only closely matched flies have to go through their entire aggressive repertoire to determine who's the champ.

Recent work by Kravitz's team sheds further light on how flies form and maintain hierarchical relationships. When flies that had lost their first fight reentered the ring after a 30-minute time-out, they almost never won. First-time losers had a 0-5-5 (win-loss-draw) record in rematches with their first opponent and a similarly feeble 0-6-6 record against naïve opponents who'd never fought another fly, Kravitz and colleagues reported in the 16 November 2006 *PNAS*. First-time losers lunged less and retreated more in their second fights, and they rarely made the first move; they only managed wins against other losers.

The researchers also found that flies appear to remember not just the outcome of their first fight but also the opponent. In second fights, familiar opponents had fewer aggressive encounters than did unfamiliar opponents. First-time losers tried out a few more lunges early on in fights against unfamiliar winners than in fights with the fly they'd lost to previously.

Now Kravitz and colleagues are hunting for changes in gene expression that may underlie the memory of past battles. Many researchers have investigated learning and memory in flies in relatively simple classical conditioning experiments, Kravitz points out. "But the learning we see happens during a social experience, and we want to know if the same genes are involved and whether we can see differences in gene expression that accompany becoming a winner or becoming a loser."

Girl fights

Like males, female fruit flies don't shy away from conflict. They may not be as easily provoked as males, but given a dab of delicious yeast to fight over, a pair of females will do their worst. ("They might be interested in headless males," Kravitz says. "We haven't looked.") Although males and females employ some common moves, female fights never escalate to "boxing" and "tussling" (a barroom-brawl mix of holding, punching, and rolling around on the ground) as do the most intense fights between males, Kravitz and colleagues reported in *PNAS* in 2004. Instead, females frequently head butt and shove—tactics rarely used by males. Females also showed no evidence of dominance hierarchies. Unlike fights between males, in which a clear victor typically emerges, fights among females seesaw indefinitely.

More recently, Kravitz's team has begun to investigate the genetics behind these gender differences. The group's initial experiments have focused on a gene called *fruitless* (*fru*) that has long been studied for its role in determining sex-specific courtship behavior. The *fru* gene is spliced differently in males and females, creating distinct messenger RNA transcripts. The male transcript can be used to make protein, but the female transcript apparently cannot. In 2005, Barry Dickson of the Research Institute of Molecular Pathology in Vienna, Austria, and colleagues reported in *Cell* that female flies genetically altered to make the male version of *fru* performed courtship behaviors usually seen in males and courted other females (*Science*, 3 June 2005, p. 1392). Male flies given female *fru* barely courted at all.

The *fru* gene has a similar effect on fighting styles, Kravitz, Dickson, and colleagues reported in the December 2006 issue of *Nature Neuroscience*. Males with the female version of *fru* were more likely to fight females than to court them. The altered males also fought like females, using head butts and shoves; they never boxed. In addition, males with female *fru* did not appear to form dominance relationships with other males. Conversely, female flies with the male version of *fru* tended to fight like males. Overall, the findings suggest that *fru* establishes the neural circuitry for aggressive behavior, just as it does for courtship behavior.

Links between fighting and courting aren't unique to flies, Kravitz says. One of the most basic decisions any animal has to make is how to respond to another of its kind, he says. "Is this

Float Like a Butterfly, Sting Like a Bee?

Fruit flies have a few moves that might impress Muhammad Ali. At Harvard, Sibu Mundiyanapurath (top) transfers fruit flies into a fighting arena (bottom left). Still images from videotaped fights show characteristic maneuvers such as (left to right) wing threat, fencing, boxing, and a defensive wing-threat display by a losing fly as he's chased by the victor. Movies of fly fights can be seen at these sites:

www.hms.harvard.edu/bss/neuro/kravitz/moviepage.html
www.pnas.org/cgi/content/full/0404693101/DC1
www.nature.com/ng/journal/v38/n9/suppinfo/ng1864_S1.html

CREDITS (TOP TO BOTTOM): G. MILLER/*SCIENCE*; CHEN *ET AL.*, *PNAS* **99**, 8 (2002); JUPITER IMAGES

someone I want to court or someone I want to fight?" Kravitz's lab now hopes to identify the neural circuitry and chemical signals underlying such decisions by expressing female *fru* in specific subsets of neurons in male flies.

Bred for battle

Other labs have taken a different approach to studying aggression in *Drosophila*. Last September, two research teams reported breeding flies to be hyperaggressive. In one study, geneticists Ralph Greenspan and Herman Dierick of the Neurosciences Institute in San Diego, California, selected aggressive flies by introducing 120 males and 60 virgin females into an enclosure with 11 small cups filled with fly food. Males' first priority was mating, but after that they settled down on the food cups and started defending their territories.

In most encounters between males, one fly was clearly dominant from the beginning and would chase any intruders on his cup, Dierick says. But a few would stand their ground and fight back. These males are the most interesting, in Dierick's view. "The real question to me is what happens when a male decides to reciprocate?"

To get at that question, he extracted these dauntless flies from the fight cage and mated them with random females from the same generation. Then he started the process all over. After 21 generations, he'd created a superaggressive line of flies that were quicker to fight and fought longer and more intensely than a line of flies created by selecting random males from the fight cages. Next, Dierick used DNA microarrays to look for changes in gene expression in the aggressive flies. In this strain, 42 genes had increased or decreased their activity by 25% or more, Dierick and Greenspan reported in the September 2006 issue of *Nature Genetics*. These genes, they noted, have diverse roles, including muscle contraction, energy metabolism, and cuticle formation.

One gene in particular, *Cyp6a20*, has stood out so far as having a potentially significant influence on aggressive behavior. *Cyp6a20* was less active than normal in the aggressive line of flies, and deactivating it in a normal strain made the flies more aggressive. The gene encodes an enzyme that plays a role in many physiological processes, including pheromone signaling, and Dierick suspects that an underactive *Cyp6a20* gene makes flies more aggressive by making them hypersensitive to pheromones.

In the September 2006 issue of *PLoS Genetics*, a team led by Trudy Mackay of North Carolina State University in Raleigh reported the results of an attempt to pinpoint genes related to aggression in their own line of hyperaggressive flies. Mackay's group identified a much larger set of candidate genes—nearly 1500—and has so far found 15 that alter aggressive behavior when mutated. As in Greenspan and Dierick's study, the candidate genes covered a wide range of physiological functions.

One puzzle is that neither set of experiments turned up genes related to serotonin, the neurotransmitter with the longest legacy in the literature on aggression. One explanation, Dierick suggests, is that the breeding experiments didn't enhance (or repress) serotonin-related genes because there was little variation in these genes in the starting populations. Going forward, he says, establishing whether serotonin plays a role in fly aggression will be important for evaluating how applicable fly studies are for understanding aggression in other animals.

The broader implications of this work on fighting flies remains an open question. "It's far too early to speculate on what these studies might tell us about vertebrate aggression," cautions Hoffmann. Kravitz is more optimistic. Genes shape complex behaviors such as aggression in all animals, he notes. "If we understand how that happens in flies, it will give us some real information about how it might happen in other animals." **–GREG MILLER**

AGBIOTECH

GM Technology Develops in the Developing World

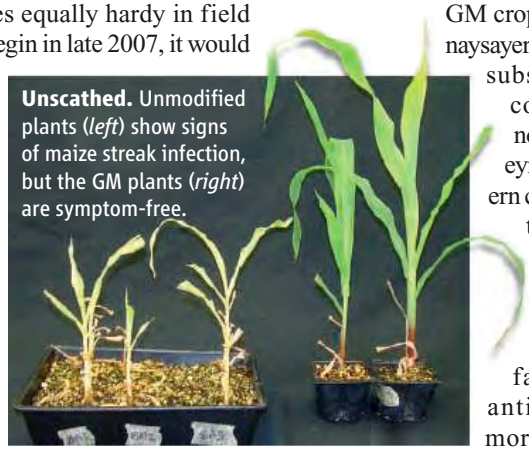
The first genetically modified crop developed entirely in Africa is gearing up for field trials. Its success would be a milestone

About 100 km north of Durban, South Africa, in a greenhouse chamber no larger than a walk-in closet, Frederik Kloppers clips a slender vial to a baby maize plant's new leaf. Inside the tube sits an insect with a potentially deadly bite, at least deadly to corn. This African leafhopper (*Cicadulina mbila*) carries maize streak virus, a scourge endemic to sub-Saharan Africa that devastates fields. Kloppers, a plant pathologist and technical manager at Pannar Seeds in Greytown, South Africa, gathers a dozen more tubes from the insect house and clips them to additional plants. Tomorrow, after the bugs have eaten their fill, he'll remove the tubes and then wait.

The fruit of more than a dozen years of effort, these maize plants have been genetically altered to resist infection by the virus. In greenhouse studies so far, the plant is highly resistant. If it proves equally hardy in field trials scheduled to begin in late 2007, it would be a milestone: the first-ever genetically modified (GM) crop developed by Africans for Africa.

But Kloppers and the plant's inventors, microbiologist Jennifer Thomson, virologist Edward Rybicki, and collaborators at the University of Cape Town (UCT), have much larger goals in mind. In a region where chronic hunger is the norm, GM maize could help alleviate grain shortages and potentially even boost economic development, says Thomson. And because plans call for selling the seed to small-scale and subsistence farmers for minimal profit, the inventors also hope it will help burnish the dim reputation of GM technology.

None of that is assured, Thomson and Rybicki concede. The plant could still fail in the field, as other African GM crop varieties such as sweet potato and cassava have done. The failures not only have disappointed the technology's advocates, but they've also fanned the flames of anti-GM sentiment. Although South Africa is one of the few African countries to permit farmers to plant GM crops within its borders, naysayers there, who still have substantial clout, have condemned the technology as a mere moneymaking tool for Western companies. Moreover, they remain unconvinced that homegrown efforts such as UCT's maize will succeed. Another failure would give anti-GM groups even more ammunition. The



Unscathed. Unmodified plants (*left*) show signs of maize streak infection, but the GM plants (*right*) are symptom-free.

CREDIT: DIONNE SHEPHERD/UNIVERSITY OF CAPE TOWN

stakes are high, and the UCT scientists are treading carefully.

The problem

Maize is not native to Africa. It likely sailed across the Atlantic from the New World as cargo during the early 1500s, according to historian James McCann of Boston University. Maize flourished and displaced other native crops during the 20th century because it grows in only a few months and requires relatively little labor—one pass of the plow instead of the three or four necessary for crops such as sorghum and millet. In sub-Saharan Africa, maize has become the staple food; it makes up more than 50% of calories in local diets. In Malawi alone, maize occupies 90% of cultivated land and accounts for 54% of Malawians' caloric intake.

Maize streak virus is likely homegrown, say scientists. It lives in native grasses. At some point, the virus adapted itself to maize and is now able to jump between grasses and corn through the bite of an infected leafhopper, which itself isn't sickened by the virus.

Like any other infection, the wrath of maize streak waxes and wanes with different environmental conditions. Some years, crop losses are minimal. But in bad years, such as 2006, it can wipe out from 5% to 100% of a farmer's maize crop.

For the past 25 years, African crop scientists have been trying to breed resistant maize by crossing plants that carry some degree of natural resistance. But the task has not been wholly successful. The trait is conferred by several genes on different chromosomes and isn't consistently transmitted to the next generation. "It's not quite clear how resistance genes are inherited," says Kloppers of Pannar Seeds. Moreover, traditionally bred varieties do not completely resist the virus, Kloppers explains. Many tolerate an infection but still produce stunted or deformed cobs.

A solution

In 1988, when Thomson took over as head of microbiology at UCT, GM technology seemed a perfect solution. Rybicki's plant virology group there was already intensively studying the virus. Perhaps they could engineer a way to stop it in its tracks?

The design seemed simple enough: The team studied the proteins necessary for the virus to replicate. If they inserted a mutated viral gene into the plant, which in turn expressed a mutated protein necessary for the virus to replicate at very high levels, it could beat out the virus's normal protein and immobilize the virus, they reasoned.

CREDIT: DIONNE SHEPHERD/UNIVERSITY OF CAPE TOWN

Devastation. Transmitted by the bite of a leafhopper, maize streak virus devastates maize fields across Africa.

But getting the genes in proved tough, Thomson says. The UCT team first tried infecting maize with a widely used vector, *Agrobacterium tumefaciens*, carrying the genes, but to no avail. Ultimately, they successfully shot DNA into the plant using a gene gun. The GM maize plant carries a mutated form of a gene from the maize streak virus and two additional regulatory genes, one derived from maize itself and another from *Agrobacterium.*

Into the field

That was 6 years ago. Since then, the UCT scientists have been working closely with Kloppers at Pannar Seeds to test the plant's hardiness against infection. Kloppers has bred a previous version of the plant that carried an antibiotic-resistance gene through four generations. So far, it resists infection consistently. Moreover, the trait appears to be inherited in a dominant fashion.

Kloppers is repeating the experiment with a new group of plants that, because of environmental safety concerns, no longer carry an antibiotic-resistance gene. He expects to carry on crossing and checking inheritance and resistance through the next few months. Provided there are no major setbacks, he expects to apply for field trials during the latter part of this year.

Field trials are crucial to assess environmental and health risks, says Dionne Shepherd, a UCT postdoc who has been working on the project for the past 10 years. The scientists plan to examine whether the crop affects soil microorganisms and also whether it affects insects that feed on it. Other studies will also ensure that the added protein is indeed digestible and not an allergen.

If all goes well, the resistant maize will be the first GM crop to be field-tested in South Africa; to date, all GM crops planted in the country have been developed and tested elsewhere. The government is now developing its own expertise to evaluate environmental and human safety, says Shepherd, and because "UCT's maize is the most advanced locally produced GM product, they want to use our plant as a guinea pig," she adds.

To avoid the pitfalls that have beset other African GM crop varieties, the UCT scientists and Pannar have been working with regulators all along. At stake, they say, is not only their crop's fate, but also the technology's reputation.

A few years ago, Kenyan scientist Florence Wambugu, who was trained and supported by Monsanto, developed a sweet potato plant resistant to the feathery mottle virus. But when scientists field-tested the crop, traditionally bred resistant varieties outperformed it. Other efforts have also stumbled during field tests. Just a few months ago, scientists at the nonprofit Donald Danforth Plant Science Center in St. Louis, Missouri, announced that cassava plants genetically modified to resist cassava mosaic disease lost the trait after a few generations.

Both setbacks have fueled ongoing skepticism about GM technology. "All this talk about the technology's benefit for Africa is just a lot of PR hype to garner funding," says Mariam Mayet of the African Centre for Biosafety, an anti-GM lobby group in Richmond, South Africa. Most of the GM crops in the world are grown for animal feed or go toward food aid, Mayet says. "The benefit mainly goes to industrial agriculture, not to small-scale farmers."

Because UCT's maize is homegrown and was supported with very little corporate money—Pannar was the project's only corporate contributor—Thomson and Rybicki hope it can dodge some of these criticisms. Private foundations that typically give money with no strings attached and the South African government funded the project's bulk. To recoup its share of investment, Pannar expects the seed to cost no more than 15% higher than non-GM seed, says Kloppers. Small-scale or subsistence farmers would likely be charged much less, he adds.

If UCT's plant succeeds, it would be the first GM crop developed by a developing country. But Africans might not be the only beneficiaries. It might also become the poster child of what many argue is a useful and important technology—and for better or worse, one that desperately needs a public relations makeover.

–GUNJAN SINHA

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LETTERS

edited by Etta Kavanagh

PTSD and Vietnam Veterans

IN HIS PERSPECTIVE "PSYCHIATRIC CASUALTIES OF WAR" (18 AUG., P. 923), R. J. MCNALLY NOTES THAT a new study by B. P. Dohrenwend *et al.* ("The psychological risks of Vietnam for U.S. veterans: a revisit with new data and methods," Reports, 18 Aug., p. 979) revised downward from 15.2 to 9.1% the rates of chronic posttraumatic stress disorder (PTSD) from the Vietnam War estimated by the National Vietnam Veterans' Readjustment Study (NVVRS). He notes that this "confirmed the suspicions of the skeptics" but fails to observe that the new study confirmed that the 2.2% prevalence rate reported by the U.S. Centers for Disease Control (CDC) (*1*) was a serious underestimate.

In numbers, this new rate means that 236,000 veterans currently have PTSD from the Vietnam War, an enormous long-term emotional and human cost of war. Recently, the director of the National Center for PTSD warned about the "psychiatric cost" of deployment in war zones, noting that we "underestimate the eventual magnitude of this clinical problem" (*2*). The Ex-Services Mental Welfare Society "Combat Stress" group in the United Kingdom saw 944 new referrals last year, an increase of 40% in recent years (*3*). The average period between discharge from the military and first contact was 12.7 years.

McNally cited a study (*4*) of 100 treatment-seeking veterans, claiming that only 41% of them had documented "combat exposure." Another 52% had clearly served in Vietnam, but "combat exposure status (was) unclear (20)" or there was "no evidence of combat exposure (32)" [(*4*), table 1, p. 469]. Given the general unreliability of military records in a war zone, the old statistical rule that "absence of proof is not proof of absence" applies. We want to stress that the nature of modern warfare, evident in the current news, is such that danger and destruction do not occur only in places designated as "combat zones."

Lastly, in addition to Dohrenwend *et al.*'s valuable service, we think it is time that scientists design studies to increase the accuracy of our prevalence estimates by applying the knowledge of over two decades of research that includes measures of biomarkers. Studies like Dohrenwend *et al.*'s in combination with new knowledge about neurobiological correlates of PTSD will contribute to science and help us to plan effectively to treat the true costs of war.

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I WISH TO CORRECT A MISCHARACTERIZATION OF my position that R. J. McNally made in his Perspective, "Psychiatric casualties of war" (18 Aug., p. 923). McNally stated that, in a column I wrote (*1*) as president of the International Society of Traumatic Stress Studies (ISTSS), I "urged critics to muffle their dissent, lest the intensity of scientific controversy distract us from attending to the needs of trauma victims." I did not say that we should stifle critics or scientific dissent. I specifically stated that "research and treatment ideas benefit from being subjected to the crucible of criticism via the scientific method" (*1*). As someone who has been conducting traumatic stress research for almost 30 years, I have consistently argued that good research is the best way to resolve controversial policy issues and that researchers also have a duty to report research results responsibly and accurately (*2*).

McNally's Perspective did not provide a balanced assessment of B. P. Dohrenwend *et al.*'s findings ("The psychological risks of Vietnam for U.S. veterans: a revisit with new data and methods," Reports, 18 Aug., p. 979), which refuted most of the prior criticisms of the National Vietnam Veterans' Readjustment Study (NVVRS). Instead, McNally focused on a misleading comparison of PTSD prevalence estimates for the entire NVVRS sample with those obtained from a clinically assessed subsample of the NVVRS that used extremely conservative criteria to determine PTSD status. Dohrenwend *et al.*'s findings show that NVVRS critics [e.g., (*3*–*5*)] were wrong when they argued that only veterans in combat roles could experience war zone stressors sufficient to produce PTSD and that veterans' reports of exposure to war zone stressors could not be independently verified.

McNally states that Frueh *et al.* (*6*) consulted "the same archival sources" as Dohrenwend *et al.* However, Dohrenwend *et al.*'s verification procedures were much more rigorous than Frueh *et al.*'s. McNally also stated that Frueh *et al.* were only able to verify combat exposure in 41% of veterans. This is true but misleading in that 93% of veterans had documented service in Vietnam. Dohrenwend *et al.*'s findings suggest that exposure to war zone stressors, not

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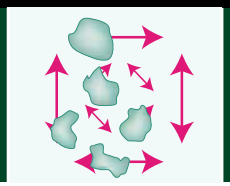

Supernova explosions

193

Deep sea symbiosis

198

just combat stressors, increases risk of PTSD, so the latter percentage is more applicable than the former.

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THE REPORT "THE PSYCHOLOGICAL RISKS OF Vietnam for U.S. veterans: a revisit with new data and methods" by B. P. Dohrenwend *et al.* (18 Aug., p. 979) and the accompanying Perspective "Psychiatric casualties of war" by R. J. McNally (18 Aug., p. 923) confirm what many in the academic community suspect about the epidemiological estimates of posttraumatic stress disorder (PTSD) generated by the National Vietnam Veterans' Readjustment Study (*1*): that the estimates are unreasonably high and uncorroborated by other scientific evidence. Indeed, a less frequently cited study commissioned by the U.S. Centers for Disease Control (CDC) yielded a modest estimate of PTSD in veterans (*2*). Looking at the data presented by Dohrenwend *et al.* (Table 1), one might argue that the new estimates are still high if "impairment" is a key criterion in defining disability.

Missing from the analyses of Dohrenwend *et al.* and McNally is the concern that PTSD as a construct is not well supported by data reduction techniques such as principal components analysis. Also absent from the discussion was the fact that clinical trials with civilians reveal that psychiatric symptoms secondary to trauma, when present, are quite responsive to treatment; yet, the largest treatment outcome study commissioned by the Department of Veterans Affairs (VA) revealed almost no salutary impact on symptoms in veterans with PTSD (*3*). Research on the effects of the VA disability system suggests that the current treatment and disability programs for PTSD promote disincentives for veterans to work toward functional outcomes, while at the same time promoting incentives to report psychiatric symptoms (*4*). It may be time for the VA to disassemble its current PTSD programs and embrace programs that promote rehabilitation and functional outcomes rather than disability. Our returning veterans deserve the promise of a productive future beyond a disability paycheck. Such a change will not be politically popular, but it will be scientifically defensible.

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IN THEIR REPORT "THE PSYCHOLOGICAL RISKS of Vietnam for U.S. veterans: a revisit with new data and methods" (18 Aug., p. 979), B. P. Dohrenwend *et al.* provide startling new data from their re-analysis of the National Vietnam Veterans' Readjustment Study (NVVRS), which indicate surprisingly little current impairment in Vietnam veterans with lifetime or current combat-related posttraumatic stress disorder (PTSD). The measure of functioning they used was a 9-point Likert-scale clinician rating, ranging from a high level of functioning, that is, "good functioning in all areas" to the lowest level, that is, "persistent danger to self or others." The scale is heavily skewed toward identifying impairment—all but the highest value represent some degree of compromised functioning—yet very few of the veterans were rated at the lower end of the scale.

Among veterans with lifetime (but not current) PTSD, over 90% were rated within the top three categories of functioning; none were rated in any of the four lowest categories. As Dohrenwend *et al.* note, this level of functioning was as good as that of the "no-PTSD" group. It seems appropriate to conclude that veterans who recovered from PTSD were functioning approximately at levels comparable to the general population.

Veterans with current PTSD at the time of the survey evidenced greater functional impairment. However, even among these current cases, only a small minority (7.4%) were rated in any of the four lowest categories of functioning; the majority (55.6%) experienced only slight impairment or some difficulties in role functioning.

Disability claims and payments to the Department of Veterans Affairs (VA) for combat-related PTSD, predicated on severe functional impairment, have risen dramatically and asymmetrically since 1999 (*1*). These new data suggest that the VA is further justified in its current review, in consultation with the Institute of Medicine, of its existing disability policies and procedures.

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Response

ACCORDING TO DOHRENWEND *ET AL.*'S reanalysis, the National Vietnam Veterans' Readjustment Study (NVVRS) (*1*) overestimated the prevalence of posttraumatic stress disorder (PTSD) by 40%, thereby confirming the central claim of its critics. Moreover, contrary to what Kilpatrick states, Dohrenwend *et al.* did not use "extremely conservative criteria to determine PTSD status." Instead, they accepted a case as PTSD-positive if the veteran received a score from one through seven on the nine-point Global Assessment of Functioning (GAF) scale. Nine is the highest possible level of functioning, whereas one is the lowest. The typical (apparent) PTSD case received a GAF score of seven, defined as "[s]ome difficulty in social, occupational, or school functioning, but generally functioning pretty well, has some meaningful interpersonal relationships OR some mild symptoms (e.g., depressed mood and mild insomnia, occasional truancy, or theft within the household)" [(*2*), p. 2]. Clearly, a seven does not indicate clinically significant impairment, as noted by Buckley. Had they been slightly more stringent (i.e., GAF rating from one through six), the prevalence would have dropped by 65%, not 40%. Thus, the estimate for current (late 1980s) prevalence would have been 5.4%—substantially lower than either Dohrenwend *et al.*'s estimate of 9.1% or the original NVVRS estimate of 15.2%. But even this

figure is more than twice as high as the 2.2% rate reported by the U.S. Centers for Disease Control (CDC) (*3*). The flawed methods of the CDC study almost certainly underestimated the true prevalence of PTSD, as emphasized by Vermetten *et al*. and many other PTSD experts (as I mentioned in my Perspective).

Kilpatrick misunderstands other criticisms of the NVVRS. The critics never claimed that self-reports of traumatic events could not be independently verified; they complained that the NVVRS researchers had not verified them. Moreover, the critics never claimed that only those serving in combat roles could be exposed to PTSD-inducing danger. Rather, the critics were puzzled how 53.4% of male veterans could develop either partial or full-blown PTSD when only between 12.5% (*4*) and 15% (*5*) had served in direct combat roles (e.g., infantry rifleman) and when the vast majority of individuals exposed to traumatic events, including combat (*6*), do not develop PTSD (*7*). As everyone, including me (*8*), acknowledges, those serving in other capacities sometimes got in harm's way. Nevertheless, as the dose-response effect implies, combat infantrymen were more at risk than supply clerks working at airbases.

To corroborate reports of trauma exposure, Dohrenwend *et al.* used a range of indicators, most of which were obtainable from veterans' DD-201 personnel files. When the DD-201 was ambiguous, they consulted additional archival sources. Using the DD-201 files, Frueh *et al*. (*9*) corroborated the trauma reports of only 41% of 100 men recently seeking treatment for PTSD. Although Vermetten *et al*. question the adequacy of military records, Dohrenwend *et al*. found the DD-201 to be a very useful corroborative source. It is unclear whether further archival inquiry would have increased the number of corroborated cases in Frueh *et al*.'s study. Frueh *et al*. found that many uncorroborated cases reported events seemingly inconsistent with their DD-201 file (e.g., uncorroborated cases reported exposure to battlefield atrocities at twice the rate of corroborated cases).

Finally, Kilpatrick says that I mischaracterized his views as expressed in his essay entitled "Our Common Bonds" (*10*). Likening our field to a "family" that often quarrels, Kilpatrick surmises that trauma victims, whose welfare constitutes our common bond, "would rather see us work together than to squabble and bicker." And despite his mentioning the importance of critique in science, he contradicts himself in his take-home message: "In my view, our field would do well to focus more on our common bonds and less on our differences." But if we downplay our differences, muffle our dissent, or curb our critique, studies like Dohrenwend *et al*.'s might never get launched.

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Response

IN HIS PERSPECTIVE ON OUR REPORT, McNally nominated as our "most newsworthy" finding the discrepancy between our somewhat lower rates of posttraumatic stress disorder (PTSD) and the original National Vietnam Veterans' Readjustment Study (NVVRS) rates (*1*). Buckley commends us for findings that he suggests indicate that the NVVRS estimates "are unreasonably high and uncorroborated by other scientific evidence." This choice of emphasis is highly selective and ignores considerations that are more important than our differences with the original NVVRS rates.

First, the discrepancies are attributable to differences in the definitions of disorder rather than to inflationary measurement error in the original NVVRS rates. To estimate rates of first onsets of war-related PTSD and rates of these onsets that were current at follow-up 10 to 12 years after the war, we used diagnostic histories obtained by experienced NVVRS clinicians from a subsample of the veterans. By contrast, self-report symptom scales were used in the full-sample NVVRS measure to provide a less time-consuming and expensive approximation of current PTSD. This approximation did not specify whether or not PTSD was war-related. However, if you take the NVVRS rate of 2.5% current PTSD for veterans who did not serve in Vietnam as an estimate of non–combat-related current PTSD, double it as per the 2:1 ratio of lifetime to current PTSD (see our Table 2 and the original NVVRS rates showing this ratio), and subtract the resulting rates from the original NVVRS 30.9% lifetime and 15.2% current PTSD rates, the result is 25.9% lifetime and 12.7% current war-related PTSD. These rates are very close to our war-related PTSD rates before adjustments for impairment and documentation of exposure (see our Table 2). This correspondence is what you would expect if, as was its aim, the NVVRS symptom scales were successfully calibrated against the subsample diagnoses.

Second, skepticism about the NVVRS rates has been stimulated by a number of factors: the discrepancy with much lower rates reported in a CDC study (*2*), a belief that only 15% of Vietnam veterans saw combat [e.g., (*3*)], and the related assumption that many veterans were either fabricating their combat experiences or that the dose-response relation between self-reports of exposure and PTSD risk was due to recall bias [e.g., (*4*–*6*)]. We pointed out that the CDC measure grossly underreports diagnosable PTSD [(*7*), Appendix E]. We demonstrated that the prevalence of combat exposure was much higher than 15% in this "war without fronts" (*8*). We found little evidence of fabrication. We showed that our record-based measure of severity of exposure to war-zone stressors, which is independent of veterans' reports of their combat experiences, is positively associated with self-reported exposure and with clinical diagnoses of PTSD. The dose-response relationship with this record-based measure of exposure is strong evidence of the validity of war-related PTSD and, we think, our most important finding.

When the NVVRS was conducted, a PTSD diagnosis did not require, as it does now, the presence of impaired functioning. Skeptics speculated that the PTSD symptoms measured in the NVVRS might indicate relatively mild psychological distress rather than true disorder [e.g., (*9*)]. The subsample diagnoses included ratings of severity and impairment useful for addressing this question. Frueh interprets our findings on functioning as indicating "surprisingly little current

impairment" among veterans with war-related PTSD. However, our results in Table 1 are for impairment at time of diagnosis, 10 to 12 years after the war. As we show, the large majority of war-related PTSD involved substantial impairment when the disorder was at its worst, even for veterans whose onsets had remitted (our Report and SOM text). We concluded that the Vietnam War took a severe psychological toll on U.S. veterans.

An epidemiological study like ours cannot speak to the treatment and compensation issues raised by Buckley and Frueh. Follow-up of the NVVRS sample could, however, provide longitudinal information on the natural history of this disorder that would be invaluable for our understanding of the nature of war-related PTSD and the factors that reduce its psychological costs.

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Disbelievers in Evolution

IN THEIR POLICY FORUM "PUBLIC ACCEPTance of evolution" (11 Aug., p. 765), J. Miller, E. Scott, and S. Okamoto show that Americans are less likely to accept evolution than citizens of other industrial nations, and that U.S. attitudes are strongly tied to fundamentalist religious beliefs. This replicates earlier results (*1*). They hint that American views on evolution may be related to political liberalism and conservatism.

The validity of their conjecture can be seen in earlier surveys. In 1993, 1994, and 2000, the General Social Surveys asked how true is the statement, "Human beings evolved from earlier species of animals." Of 3673 American respondents offering an opinion, a majority (53%) called the statement definitely or probably not true (*2*). Respondents also reported their political views, ranging from extremely liberal to extremely conservative. Political liberals were significantly more likely than conservatives to believe that humans evolved.

In Fig. S1 (*3*), the percentage of respondents believing in human evolution is plotted simultaneously against political view (conservative, moderate, liberal), education (high school or less, some college, graduate school), and respondent's religious denomination (fundamentalist or not) (*2*). Belief in evolution rises along with political liberalism, independently of control variables.

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TECHNICAL COMMENT ABSTRACTS

COMMENT ON "A Common Genetic Variant Is Associated with Adult and Childhood Obesity"

Christian Dina, David Meyre, Chantal Samson, Jean Tichet, Michel Marre, Beatrice Jouret, Marie Aline Charles, Beverley Balkau, Philippe Froguel

Herbert *et al*. (Reports, 14 April 2006, p. 279) reported an association between the *INSIG2* gene variant rs7566605 and obesity in four sample populations, under a recessive model. We attempted to replicate this result in 10,265 Caucasian individuals, combining family-based, case-control, and general population studies, but found no support for a major role of this variant in obesity.

Full text at www.sciencemag.org/cgi/content/full/315/5809/187b

COMMENT ON "A Common Genetic Variant Is Associated with Adult and Childhood Obesity"

Ruth J. F. Loos, Inês Barroso, Stephen O'Rahilly, Nicholas J. Wareham

Herbert *et al*. (Reports, 14 April 2006, p. 279) found that the rs7566605 genetic variant, located upstream of the *INSIG2* gene, was consistently associated with increased body mass index. However, we found no evidence of association between rs7566605 and body mass index in two large ethnically homogeneous population-based cohorts. On the contrary, an opposite tendency was observed.

Full text at www.sciencemag.org/cgi/content/full/315/5809/187c

COMMENT ON "A Common Genetic Variant Is Associated with Adult and Childhood Obesity"

Dieter Rosskopf, Alexa Bornhorst, Christian Rimmbach, Christian Schwahn, Alexander Kayser, Anne Krüger, Grietje Tessmann, Ingrid Geissler, Heyo K. Kroemer, Henry Völzke

Contrary to the findings of Herbert *et al*. (Reports, 14 April 2006, p. 279), homozygous carriers of the C allele of the rs7566605 variant near the *INSIG2* gene did not exhibit a significantly increased risk for obesity in a large population-based cross-sectional German study. A subgroup analysis, however, revealed that this allele significantly increased the risk for obesity in already overweight individuals.

Full text at www.sciencemag.org/cgi/content/full/315/5809/187d

RESPONSE TO COMMENTS ON "A Common Genetic Variant Is Associated with Adult and Childhood Obesity"

Alan Herbert, Norman P. Gerry, Matthew B. McQueen, Iris M. Heid, Arne Pfeufer, Thomas Illig, H.-Erich Wichmann, Thomas Meitinger, David Hunter, Frank B. Hu, Graham Colditz, Anke Hinney, Johannes Hebebrand, Kerstin Koberwitz, Xiaofeng Zhu, Richard Cooper, Kristin Ardlie, Helen Lyon, Joel N. Hirschhorn, Nan M. Laird, Marc E. Lenburg, Christoph Lange, Michael F. Christman

Identification of genetic variants affecting complex traits such as obesity is confounded by many types of bias, especially when effect sizes are small. Given our findings of a positive association between rs7566605 and body mass index in four out of five separate samples, a false positive finding cannot be ruled out with certainty but seems unlikely. Meta-analyses of multiple large studies will help refine the estimate of the effects of rs7566605 on body mass index.

Full text at www.sciencemag.org/cgi/content/full/315/5809/187e

CORRECTIONS AND CLARIFICATIONS

Reports: "Relating three-dimensional structures to protein networks provides evolutionary insights" by P. M. Kim *et al.* (22 Dec. 2006, p. 1938). In note 32, the funding acknowledgment should read, "This work was supported by NIH grants N01-HV-28186 and RR19895." Additionally, on page 1939, a maximum degree of 14 was reported for the SIN v1; this number refers to an earlier version of the SIN (v0.9). The SIN v1 as reported in the paper has one node with a degree of higher than 14. All versions of the SIN and current statistics on them are available at http://SIN.gersteinlab.org.

Special Section: Breakthrough of the Year: "Minute manipulations" (22 Dec. 2006, p. 1855). This item incorrectly described Piwi-interacting RNAs (piRNAs) as binding to Piwi genes, when in fact piRNAs bind to Piwi proteins.

Perspectives: "The brain's dark energy" by M. E. Raichle (24 Nov. 2006, p. 1249). The author's affiliation was incorrect. It should be Department of Radiology, Washington University School of Medicine, St. Louis, MO 63110, USA. E-mail: marc@npg.wustl.edu.

HISTORY OF SCIENCE

Scientia Sinesis

Thomas S. Mullaney

Why was Europe the birthplace of modern science, and not China? In *A Cultural History of Modern Science in China*, Benjamin Elman boldly revisits this well-trod ground and attempts to survey it anew. As he announces in the book's introduction, Elman (professor of East Asian history at Princeton University) wishes to reposition the classically Eurocentric account of modern science. He aims to overturn the "failure narrative" that is typically invoked when trying to explain why China did not develop the fields of modern physics, chemistry, statistics, advanced mathematics, and so forth.

In this slender monograph, which doubles as the abridged version of Elman's much larger *On Their Own Terms: Science in China, 1550–1900* (*1*), the author mounts an empirically rich argument detailing a three-part process of transmission, mediation, and incorporation that shaped China's encounter with European science. In each of these three stages, a complex interplay of historical and cultural factors resulted more often than not in a checkered and turbulent transmission of scientific information from the West to China. For Elman, this choppiness partially explains the uneven development of modern science in China as compared with in Europe.

Elman's study focuses on two groups, Jesuit advisers and Protestant missionaries, whom he identifies as the primary transmitters of modern scientific knowledge from Europe to China prior to 1900. Starting in the early 1600s, the Jesuits made inroads into the imperial court by drawing on their astronomical and cartographic knowledge to answer the emperor's calls for a more accurate calendrical system and more precise maps of the empire. Protestant missionaries arrived some two centuries later, responding to the growing demand among Chinese reformers for advanced industrial and military technologies—a demand that, as Elman notes, was itself prompted by China's defeat at the hands of the British in the Opium Wars.

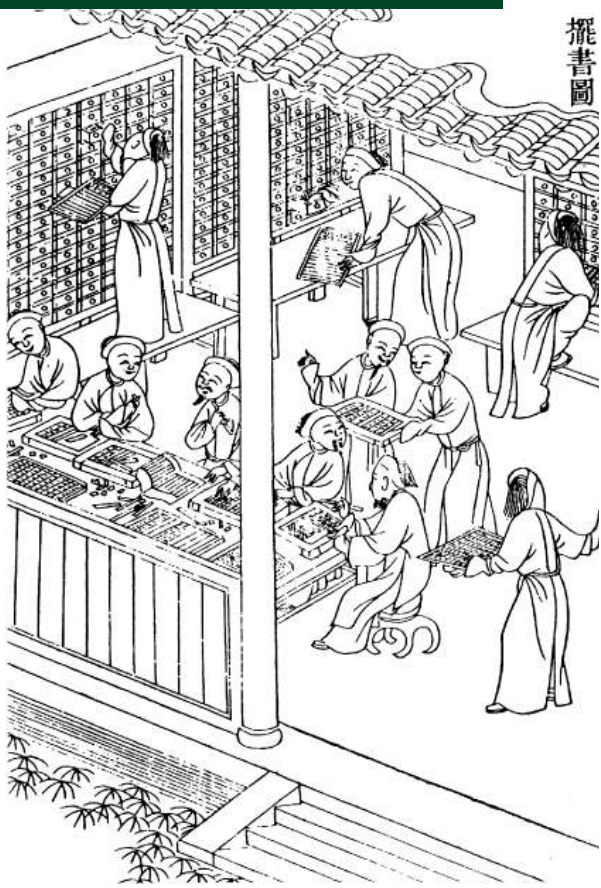
A Cultural History of Modern Science in China

by Benjamin A. Elman

Harvard University Press, Cambridge, MA, 2006. 324 pp. $35, £22.95, €32.30. ISBN 9780674023062.

These 300 years exposed Chinese intellectual circles to many of the key elements of Western science. Many of the most important theories and principles, however, did not make the journey. Channels of transmission were frequently filtered or obstructed by powerful mediating forces, particularly the religious commitments of the Jesuits and Protestants themselves. In deciding which scientific theories to convey and how to portray them, these representatives of Christianity sometimes delayed or prevented many of the most critical components of the scientific revolution from ever reaching China. As committed Aristotelians, for example, the Jesuits were remiss when it came to introducing the principles of Newtonianism, a factor that delayed the full translation of the *Principia* by over a century. Similarly, Protestant missionaries were highly selective in their portrayal of Darwinism, shaping Chinese understanding of the theory so that it would correspond as much as possible with their own creationist orientation. In other cases, the Jesuits and Protestants in China had simply lost touch with contemporaneous developments taking place back home, resulting in the not-infrequent transmission of obsolete or disproved theories, which the Chinese then mistook for cutting-edge Western science.

In addition to religious mediations such as these, the passage of modern scientific knowledge from Europe to China was further filtered and obstructed by members of the imperial court. Because the Chinese court maintained a de facto monopoly on interactions with European visitors, any given theory or technology first had to appeal to the court's sense of utility before it could be incorporated into the larger infrastructure of sanctioned knowledge. Consequently, the influx of Western science was subject to a continual litmus test of applicability, a process that favored those imports that could expeditiously resolve pressing problems of the day. Areas of abstract research, such as Leibniz's mathematical notation, received comparatively scant attention, a factor that in this case delayed China's appreciation and incorporation of the calculus.



Setting movable type in the Qianlong Imperial Printing Office (late 18th century) (*2*).

Cultural sensibilities also played a role. As Elman explains, Chinese officials were allergic to self-satisfied Western claims of scientific superiority and universality. As a check on Western arrogance, sciences such as physics, chemistry, and so forth were officially categorized under the rubric of "Western learning," in much the same way that the Western world now diminishes non–Euro-American intellectual and artistic output by dubbing it "Eastern philosophy," "world music," "traditional Chinese medicine," and so forth. In later years, the imperial court stepped up these efforts, commissioning Chinese elites to comb through the ancient classics in an attempt to prove that all the great theories of Western science were in fact merely derivative corroborations of prior Chinese discoveries.

As a whole, Elman's study is a tremendous achievement, both in its analytical insight and empirical depth. Nonetheless, it is not altogether clear whether Elman succeeds in disrupting existing historiographic frameworks. Even with his rich and nuanced analysis of Chinese scholarship, for example, his portrayal of China remains rather consistent with the classical account of modern science: China continues to function as the recipient of Western knowledge; the only questions being how speedily such knowledge was transmitted,

CREDIT: FIG. 3.2 IN *A CULTURAL HISTORY OF MODERN SCIENCE IN CHINA*

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how faithfully it was mediated, and how readily it was incorporated. Whereas Elman's account certainly expands the failure narrative to include European intermediaries such as the Jesuits and the Protestants, it does not fundamentally disrupt this narrative or provide a viable counternarrative. This observation is not so much a critique of *A Cultural History of Modern Science* as of the limitations of our collective definition of "modern science" itself—which is so firmly and uncritically tied to European intellectual output that writing anything but a Eurocentric history of it is perhaps impossible. To recenter the history of modern science, it would seem, the very concept would have to be redefined along more catholic lines.

References

1. B. A. Elman, *On Their Own Terms: Science in China, 1550–1900* (Harvard Univ. Press, Cambridge, MA, 2005).
2. This illustration originally appeared in *Qinding Wuying dian juzhen ban chengshi* (Beijing, 1776).

10.1126/science.1135703

SOCIOLOGY

Bringing Down the House to Add a (Nice) Room

Saskia Sassen

Ivan Light's *Deflecting Immigration* makes a valuable contribution, one that illuminates various trends either overlooked or left unaddressed in the standard scholarship about immigration in the United States. Where much of that scholarship sees a lineal progression, Light (a sociologist at the University of California, Los Angeles) identifies a discontinuous process, which he renders as a two-stage model: demand-driven immigration becomes supply-driven. He incorporates into his model variables, such as local policy, that are usually considered exogenous to the immigration process. In so doing, Light makes a second contribution: he shows that once immigration enters the supply-driven stage, it gets redirected to other, secondary destinations.

Empirically focusing on Los Angeles (and especially on Latino immigration), Light offers a functional analysis of how worsening economic and housing conditions for low-wage immigrants in major cities lead to intolerance of poverty on the part of the receiving community. This development of poverty intolerance depends crucially on political reasons; insofar as the receiving community does not tolerate misery and degradation beyond a certain point, local authorities' enforcement of existing housing and workplace regulations begins to reduce the housing and work opportunities of migrants. This in turn leads to outmigration and the geographic dispersal of migrants to other cities.

Deflecting Immigration
Networks, Markets, and Regulation in Los Angeles

by Ivan Light

Russell Sage Foundation, New York, 2006. 262 pp. $35. ISBN 9780871545381.

Deflection is what Light names the process by which supply-driven immigration leads to the geographic dispersal of immigrants. A very interesting and useful category, deflection functions in multiple ways, most of which are wired into the social, civic, economic, and political fabric of cities. In this view then, intolerance of the more severely disadvantaged immigrants, police harassment of informal vendors, and inspections targeting poor immigrants in informal housing are not anomalous or deviant. They are instead part of a more complex de facto policy that ensures the departure of those migrants whose access to housing and jobs is seriously compromised by the lack of opportunities (demand).

Deflection is functional because, by redistributing these migrants (who represent an excess supply from the perspective of a city's tolerance for poverty), it ensures a better survival for the departing (or expelled) migrants. It also sets a limit to the increase of poverty, misery, extreme survival economies, and informal housing in the expelling city; thus it has the additional effect of raising the chances of reasonable livelihoods for the immigrants who remain. Light estimates that in the 1990s, 1 million immigrants were deflected from Los Angeles to lower-traffic destinations where the rent-to-wages ratio was more favorable.

I found less persuasive, and actually rather incomprehensible, the somewhat unnecessary combat that Light unleashes on globalization scholars who have addressed the question of immigration—a relatively rare, albeit growing, minority. Given the small size of this group and the recurrent references, I felt directly addressed. Light begins this attack nicely enough. He posits that a policy argument of sequential absorption and deflection appears "superficially to support the globalization arguments critiqued in chapter 1" that demand drives immigration. The author then argues that, notwithstanding appearances, the globalization crowd has it wrong. He makes four rejoinders.

To support his argument, Light has to attribute some rather extreme arguments to what he identifies as the globalization view: He claims that globalization theorists expect low-wage immigrants not to move and to stay in world cities. World, or more precisely, global cities are strategic spaces for the corporate global economy; one of their key features is a growing stratum of high-income professionals, a growing stratum of low-wage jobs, and the resulting increased inequality. Immigrants have filled many of the low-wage jobs. Light supposes that globalization theorists believe that if immigrants were to be deflected to another city, that would be because those secondary destinations were or were becoming world cities. Second, Light asserts that these theorists posit that these immigrants' only jobs are household jobs for rich professionals and that they cannot find employment in manufacturing or other such fields. Third, Light writes that "the 'optimist' globalization scenario" posits that immigrants would never "exhaust" the gardening, nanny, etc. jobs for affluent professionals. Fourth, and as a result of the former, immigrants would never push cities into poverty intolerance.

In an example that captures the tone of his complaint, Light proclaims, "This indictment convicts the globalization theorists of having peddled an optimistic and saccharine expectation that developed countries could expect eternally painless immigration from the Third World. They promised a party that would never end." Although I function as exhibit number one in this series of statements, I do not recognize myself in this description. Anyone who has read my work knows that I am a critic of corporate economic globalization. I have spent much effort showing that the development of global cities has brought about increased inequality and much misery (*1*) and that politics (including at the city level) is part of good immigration policy (*2*). Light could have extracted a couple of good insights, even variables for analysis, from this globalization research. That would have added to the strengths of *Deflecting Immigration*.

References

1. S. Sassen, *The Global City: New York, London, Tokyo* (Princeton Univ. Press, Princeton, NJ, ed. 2, 2001).
2. S. Sassen, *Territory, Authority, Rights: From Medieval to Global Assemblages* (Princeton Univ. Press, Princeton, NJ, 2006), chap. 6.

10.1126/science.1136272

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SCIENCE AND LAW

The Obvious War

The U.S. Supreme Court will soon render its decision on what makes an invention obvious—a decision that will likely have significant effects.

Michael R. Samardzija*

The war is over what inventions are patentable. Although the particular patent at issue claims an adjustable pedal assembly, the parties assembling for the battle are "high tech"—semiconductor companies, software companies, telecommunication companies—versus "health care"—pharmaceutical companies, biotechnology companies, universities. Each side has professed that its very existence is at stake.

Under U.S. patent law, an inventor may obtain a patent only if the U.S. Patent and Trademark Office (USPTO) finds, among other things, the invention novel (*1*) and nonobvious (*2*). Both of these terms have specific legal meanings, which are not necessarily intuitive. An invention lacks novelty when each element of a patent claim is found in a single prior art reference. A prior art reference may be, for example, a printed publication, an oral disclosure, a public use, an offer to sell, or a sale. An invention is obvious when the elements of a patent claim are found in two or more prior art references, and "the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." (*2*).

In an effort to better explain this general standard, the U.S. Supreme Court in *Graham* v. *John Deere Co.* explained that obviousness depends on (i) the scope and content of the prior art; (ii) the differences between the claimed invention and the prior art; (iii) the level of skill of the average practitioner in the art; and (iv) any relevant secondary considerations, including commercial success, long felt but unsolved needs, and the failure of others (*3*). The reason for these Supreme Court decisions was in large part because the courts frequently invalidated patents on obviousness grounds by viewing the invention and the prior art in hindsight.

In applying the obviousness test, the Court of Appeals for the Federal Circuit ("Federal Circuit"), the appellate body with jurisdiction over patent cases, is primarily concerned with rooting out obvious inventions while specifically excluding the use of hindsight to improperly reconstruct the invention from the prior art (*4*, *5*). In this effort, the court has articulated a subsidiary requirement known as "the suggestion test," which requires the judge or jury to determine whether the prior art would inexorably lead to the claimed invention but to stay away from "20/20 hindsight" (*6*, *7*).

Gobbledygook! This is the term used by Justice Antonin Scalia of the U.S. Supreme Court during the oral arguments at *KSR International Co.* v. *Teleflex, Inc.,* to describe the current standard for defining an invention as obvious (*23*).

The battle is over whether the Federal Circuit's obviousness standard is too lax, resulting in the issuance of numerous patents of dubious validity (*8*). The Federal Circuit argues that its suggestion test is an outgrowth of applying Supreme Court directions (*7*). Opponents assert that the Federal Circuit's test has no basis in any Supreme Court case and instead flies in the face of the Supreme Court's obviousness standard (*9*). According to the "high-tech" proponents, the USPTO has continually issued patents for inventions that are merely incremental advances of the prior art, partly as a result of this lax standard. For example, through 2002, the USPTO issued approximately 90,000 patents claiming some aspect of the central processing unit (CPU), which were held by over 10,000 different entities. Commentators have argued that this "patent thicket" unavoidably contains overlapping patents (*10*). The companies face relentless accusations of patent infringement on patents that they believe have questionable validity. These suits threaten their ability to commercialize products and can extort unreasonably large sums of money to avoid the high costs of patent litigation. For example, in 1991, U.S. companies spent over $1 billion enforcing or defending against patent lawsuits, while only spending roughly $300 million on R&D expenditures (*11*). Because the high-tech allies are constantly defending themselves against patent infringement attacks, they favor increasing the patentability threshold—making obviousness easier to prove—and decreasing patent holders' ability to shut companies down and to receive large monetary damages.

To the parties that I have collectively called "health care," the patent system has been effective. Aside from some issues over the quality of patents (*12*, *13*) and the USPTO's inefficiency at issuing patents (*14*), the health-care alliance believes that the patent system generally functions well and that major changes are not only unwarranted, but would likely devastate the health care of our country. They believe that the best way to ensure investment in health care and continued advancement of medical discoveries is to maintain strong patent protection (*15*). Because the health-care allies must be able to enforce their patents, they favor the current patentability threshold and patent holders' ability to strongly enforce their patent rights.

Both sides have advocated their view to the U.S. Supreme Court, which now has the opportunity to clarify the obviousness standard in *KSR International Co.* v. *Teleflex, Inc*., currently before them. On 28

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November 2006, the Court heard argument on the specific question of "[w]hether the Federal Circuit has erred in holding that a claimed invention cannot be held 'obvious', and thus unpatentable under 35 U.S.C. § 103(a), in the absence of some proven '"teaching, suggestion, or motivation" that would have led a person of ordinary skill in the art to combine the relevant prior art teachings in the manner claimed.'" (*16*). Thus, the distilled issue before the Court is whether the Federal Circuit's suggestion test is a valid test to determine obviousness.

Teleflex and KSR both make and sell an adjustable pedal assembly for use with automobiles having engines that are controlled with an electronic throttle. Teleflex alleged that KSR infringed claim 4 of Teleflex's U.S. Patent No. 6,237,565 (the so-called '565 patent). KSR countered that Teleflex's claim was invalid because it was obvious. At first glance, the invention at issue in *Teleflex* v. *KSR* may seem obvious, as that word is commonly used in the English language. Adjustable pedals have been used in automobiles for over 30 years. Automobile makers have used electronic sensors to control the engine for more than 10 years. If adjustable pedals are combined with the use of electronic sensors, the result is an adjustable pedal that uses electronic sensors. The district court concluded that such a combination would have been obvious to one of skill in the art, and held that claim 4 was invalid (*17*).

But on appeal to the Federal Circuit, a three-judge panel vacated the district court's decision, and held that the lower court "applied an incomplete suggestion test" when it found claim 4 obvious (*18*). Specifically, the panel found that the lower court should have made "specific findings … as to a suggestion or motivation to attach an electronic control to the support bracket of the [prior art] assembly." (*19*). According to the panel, the cited prior art did not address the specific problem that the Teleflex inventors were trying to solve (*18*).

While the device covered by the '565 patent was invented to provide a smaller, less complex, and less expensive electronic pedal assembly, the prior art either was directed at solving a different problem, or actually suffered from the problem (i.e., the apparatus was bulky, complex, and an expensive electronic pedal assembly). According to the panel, the lower court did not explain how suffering from the problem specifically would have motivated one skilled in the art to make the improvement on the prior art (*18*).

The Supreme Court has not heard an obviousness case since 1976 in *Sakraida* v. *Ag Pro, Inc.* (*20*). In *Sakraida*, the Supreme Court held that a combination that only consolidates known elements without changing their functions is obvious and thus precluded from patentability. Some argue that, under the *Sakraida* rule, the *Teleflex* patent is arguably obvious because it combines known elements. The high-tech allies strongly urge the Supreme Court to revive the *Sakraida* rule. They argue that combinations of old elements should not be permitted unless they create some new "synergy" or "functionality" that was not present in the old combination. Some suggest shifting the current burden to the patent applicant to prove that her invention is nonobvious, although they do not explain how a patent applicant could meet this proposed burden of proof.

The health-care allies advocate continuing the use of the current standard. Under the *Sakraida* rule, most, if not all, new-use patents for known compounds would likely be found obvious. They argue that compounds such as Forest's drug Namenda (memantine HCl), approved in 2003 for the treatment of Alzheimer's, and ImClone's Erbitux, which had patent protection directed only to combination therapeutic treatment of colorectal carcinoma with irinotecan—all known drugs protected by method-of-use claims—would have never had their current impact had these companies not had the benefit of patent protection.

The battle centers around whether the Federal Circuit's test appropriately deals with the problem of hindsight. In their brief to the Supreme Court, a group of 24 law professors argued that the Federal Circuit's suggestion test does not solve the hindsight bias, because the test is too narrow, in that, for an invention to be considered obvious, it has to have been specifically described as a possibility in the referenced prior art. Yet two reviews of the fact patterns in past cases conclude that the Federal Circuit's test does not produce erroneous outcomes (*21*, *22*). These studies indicate that neither the Federal Circuit's test nor the Supreme Court's test completely resolve the hindsight problem.

The best solution may be in fact to retain the Federal Circuit's suggestion test along the principle of "The devil you know is better than the devil you don't." Whichever way the case is decided, the Supreme Court will disappoint one group of allies, and the effects of its decision will be felt by all in the years to come. If the Court sides with the health-care alliance, although there is no evidence that advances in software and high-end electronic devices would be negatively impacted, the associated costs of litigation, however, would likely be shifted to the consumers. If the Court sides with the high-tech alliance, however, the costs could be much higher, by negatively impacting advances in medicine and investment in biomedical research. Alternatively, the Supreme Court may surprise all, as it often does, and devise some sort of Solomonic solution no one anticipated.

References and Notes

1. 35 U.S. Code (U.S.C.) §102.
2. 35 U.S.C. §103.
3. *Graham* v. *John Deere, Co.* 383 U.S. 1, 17 (1966).
4. *Grain Processing Corp.* v. *American Maize-Products Corp.*, 849 F.2d 902, 5 USPQ2d 1788 (Fed. Cir. 1988).
5. *Gillette Co.* v. *S. C. Johnson & Son, Inc.*, 919 F.2d 720, 16 USPQ2d 1923 (Fed. Cir. 1990).
6. *SIBIA Neurosciences, Inc.* v. *Cadus Pharmaceutical Corp.*, 225 F.3d 1349, 1356 (Fed. Cir. 2000).
7. *Dystar Textilfarben GmbH & Co.* v. *C. H. Patrick Co.*, No. 06-1088, 2006 WL 2806466, pp. 8 and 9 (Fed. Cir. 3 October, 2006) [citing *Alza Corp.* v. *Mylan Labs., Inc.,* No. 06-1019, 2006 U.S. App. LEXIS 22616, pp. 7 and 8 (Fed. Cir. 6 September 2006)].
8. Examples of such dubious patents frequently identified are Amazon's one-click patent (U.S. Pat. No. 5,960,411), a 5-year-old boy's method of swinging from a swing patent (U.S. Pat. No. 6,368,227), a crustless peanut butter and jelly sandwich (U.S. Pat. No. 6,004,596), and, for those who are follicularly challenged, the three-way comb-over (U.S. Pat. No. 4,022,227).
9. P. M. Janicke, *Antitrust Law J.* **69**, 645, 661 (2002).
10. C. Shapiro, in *Innovation Policy and the Economy*, vol. 1, A. Jaffe, J. Lerner, and S. Stern, Eds. (National Bureau of Economic Research, Cambridge, MA, 2001), pp. 119–150; available at http://faculty.haas.berkeley.edu/shapiro/thicket.pdf.
11. J. Lerner, *J. Law Econ.* **38**(2), 463 (1995).
12. U.S. Patent and Trademark Office (USPTO), *Transforming to Meet the Challenges of the 21st Century: A Report by a Panel of the National Academy of Public Administration* (USPTO, Washington, DC, August 2006), p. 64.
13. The average error rate for 2004 was 5.3%.
14. It takes, on average, 36 months for the USPTO to allow a patent application (24.3 months in the semiconductor technology group, 30.4 months in the biotechnology technology group, 41.9 months in the computer architecture software technology group) (*12*).
15. Changing the obviousness standard in a way that would lower an alleged patent infringer's burden to show that the claimed patent is obvious and, therefore, invalid would weaken the patent system.
16. Docket No. 04-1350, Questions Presented; available at www.supremecourtus.gov/qp/04-01350qp.pdf (accessed 3 October 2006).
17. *Teleflex* v. *KSR*, 298 F. Supp.2d 581, 592 (E.D. Mich. 2003).
18. *Teleflex, Inc.,* v. *KSR International Co.*, No. 04-1152 (6 January 2005) (slip op.).
19. See note (*18*), at p. 11.
20. *Sakraida* v. *Ag Pro, Inc.,* 425 U.S. 273, 189 USPQ 449 (1976).
21. G. Mandel, *Yale J. Law Technol.* (in print 2006).
22. R. P. Wagner, L. Petherbridge, *Texas Law Rev.* **85**(7), in press.
23. *KSR International Co.* v. *Teleflex, Inc.*; p. 41, line 11, of www.supremecourtus.gov/oral_arguments/argument_transcripts/04-1350.pdf.

10.1126/science.1136053

ECOLOGY

Dipping into the Rare Biosphere

How can we explore the "seed-bank" of rare microbial species in marine environments?

Carlos Pedrós-Alió

When molecular methods were introduced into microbial ecology in the mid-1980s (*1*), there were two surprises: Most DNA sequences found bore no resemblance to organisms known from cell culture, and the microorganisms that could be cultured were almost never found in molecular surveys (see the figure). These findings indicated that microbial diversity is much larger than had been anticipated. It also revealed that some bacteria occur at such low abundance that molecular techniques cannot detect them. In the meantime, marine microbiology has become an extremely active and innovative field. For example, two reports in this issue, by Markert *et al.* on page 247 (*2*) and Not *et al.* on page 253 (*3*), demonstrate that current molecular techniques can suggest geographic distribution and characterize the symbiotic relationships that enable organisms to survive in extreme habitats. Morever, recent advances in sequencing technology are beginning to open our eyes to the huge dimensions of the microbial diversity hidden in nature.

I will focus on marine bacteria, although most arguments may also be valid for other microorganisms. The marine environment is the largest ecosystem on Earth, and the number of rare species of microorganisms is potentially enormous. Current estimates of the total number of bacterial species range from millions (*4*) to hundreds of millions (*5*). If we consider that a milliliter of seawater may contain 1 million bacterial cells, a very large number of species may be represented by just one cell. Extrapolate this number to the volume of the ocean and the diversity becomes unimaginably vast. Because of the enormous volume of the oceans, even species represented by billions of cells would be rare.

An important property of this rare biosphere (*6*, *7*) is that one cell of a bacterial taxon can become abundant simply by clonal replication if conditions change and become suitable for its growth. Contrast this with sexual organisms, where finding a mate becomes too improbable below a certain threshold abundance. Similarly, many small eukaryotes can also grow and divide without the need for a sexual partner and are not limited by being rare.

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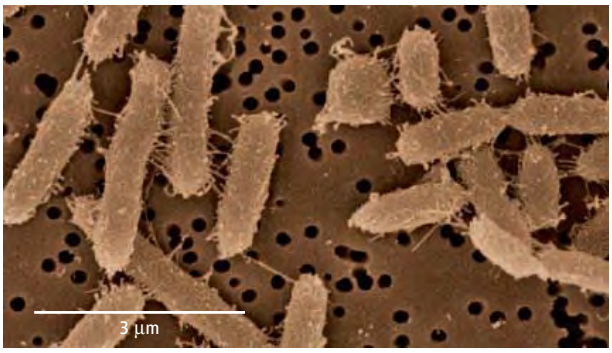

Isolating rare bacteria. The bacterium *Leeuwenhoekiella blandensis* has been isolated from the Mediterranean Sea and cultured in the laboratory. Despite several years of seasonal studies at the same spot, it has not been retrieved by molecular methods.

Another special property of the rare bacteria is that death is highly unlikely (*6*). The main causes of death for bacteria are attack by viruses that lyse the cells and predation by protists, the small heterotrophic eukaryotes that feed on bacteria or on other (generally smaller) protists. Viruses depend on encounter probabilities to find their prey, and thus if an organism is vanishingly rare, it is unlikely to be met by its specific virus. Actively growing bacteria are known to be bigger than starving bacteria, and protists selectively prey on the largest and most active bacteria (*8*, *9*). Rare taxa tend to be starving and grow slowly compared with abundant taxa. So rare taxa tend to be represented by smaller cells and will be overlooked by grazers. Therefore, the rare taxa will persist in the environment for a long time. As a result, any habitat will have a very large biodiversity, formed by a few dozen abundant taxa plus a large collection of rare taxa that current molecular methods cannot retrieve (*6*).

How can this biodiversity be explored? Pure cultures are the first approach. In principle any microorganism, irrespective of its abundance, can be retrieved provided the right selective conditions are in place. Even though many microorganisms cannot be isolated at present, recent efforts to develop novel culturing techniques show promise for the situation to improve in the near future (*10*, *11*).

A second approach is the use of polymerase chain reaction (PCR) amplification of 16*S* ribosomal RNA genes present in a sample. These are then used to construct clone libraries. Normally, a set number of clones is sequenced, and statistics are used to estimate the total number of different taxa in the library. The most careful attempts with this approach (*12*, *13*) have retrieved up to 500 taxa from marine samples, and estimates of the total diversity reach 2000 taxa in one library (*12*, *13*). We can use molecular tricks in PCR to find particular microorganisms. For example, specific primers for certain groups of microorganisms can be used in the PCR reaction, or a nested PCR can be performed (*14*, *15*). These will retrieve more rare sequences belonging to the target group. But this approach will only find the target group; it will never discover novel sequences. To probe the depths of the rare biosphere, the only solution is to sequence a massive number of clones. Venter *et al.* (*16*), for example, took a brute-force shotgun sequencing approach to analyze samples from the Sargasso Sea. This method does not use PCR, thus avoiding biases against rare sequences. The drawback is that the clone library contains every gene of all the microorganisms present in the sample. After sequencing 1 billion base pairs, Venter's team estimated they had found 1800 taxa. More powerful, cheaper sequencing approaches are on the horizon. Sogin *et al.* (*7*) have recently used a sequencing-by-synthesis approach to analyze some deep-water samples from the North Atlantic. To avoid having to sequence all genes present, they used one PCR step. Although all their sequences were relevant for diversity, they were, alas, still subject to the PCR bias. In one of their samples, the authors found 5266 different taxa, and statistical estimates indicated a total richness of 18,191 different taxa.

As these novel sequencing approaches are applied to diversity studies and the number of sequencing reactions increases to millions or hundreds of millions, we can expect the discovery of novel sequences to increase by at least another order of magnitude. The challenge is to retrieve the bigger picture behind those sequences. Can we use these genes in medicine or biotechnology? What do these sequences tell us about biogeochemical processes in the ocean? What will

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happen as climate change progresses? What do these genes and the functions they represent tell us about the evolution of life? The technology is there and the theory is in place, making this a very exciting time to be a student of microbial diversity.

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ASTRONOMY

A Supernova Riddle

Analysis of the polarization of light from supernovae can reveal the shape and distribution of matter ejected from exploding stars.

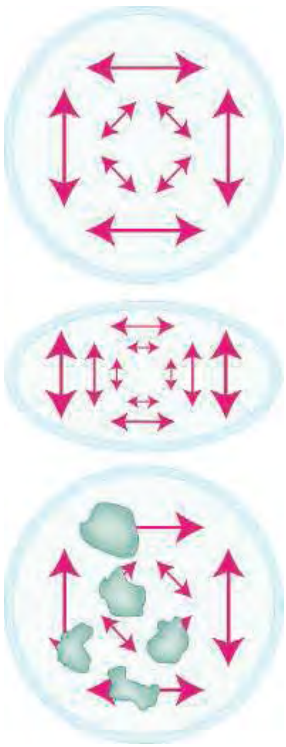
Douglas C. Leonard

Roughly once per second in the observable universe, a star explodes and announces its death with an optical display that for weeks rivals the brilliance of its parent galaxy. These supernova events are classified into several types, but among the most interesting are those called type Ia supernovae (SNe Ia). Astronomers' love affair with these beacons began in earnest about a decade ago, when two groups put them to work as distance indicators and precisely mapped the recent expansion history of the universe. Before this work, most researchers expected gravity to be slowing the expansion. Instead, the data revealed a universe expanding at an accelerating rate, a finding heralded by *Science* as the Breakthrough of the Year in 1998 (*1*) and one that has since survived intense scrutiny and complementary experimental checks. Yet for all the fanfare and empirical success, it must be acknowledged that we are fundamentally ignorant: We do not know how these stars explode. On page 212 of this issue, Wang *et al.* (*2*) identify a suggestive trend in an impressive set of SN Ia data that may point the way toward a deeper understanding of these enigmatic cosmic blasts.

Despite an embarrassing dearth of direct observational evidence, the first part of the story of SNe Ia is largely considered settled. Each future SN Ia begins as a carbon-oxygen white dwarf—the compact corpse of a low-mass star like our Sun after its nuclear-burning life is over—accreting matter through some mechanism (mass flow from the envelope of a close companion star seems most likely) until a critical central density is achieved and a thermonuclear runaway is triggered. There is general agreement that, once initiated, the burning front progresses through the star for a time as a subsonic deflagration. But at this point in the story, harmony ends and pitched battles begin, with some favoring an enduring deflagration front and others insisting on a transition to a supersonic detonation.

The most recent "delayed detonation" models appear to better match observed SNe Ia: The events produced in these simulations are bright enough (a perennial problem for deflagration models) and have the proper ejecta composition and stratification (*3*). The mechanism that triggers the deflagration-detonation transition remains a mystery, however, and so the pure deflagration model still retains its share of adherents. In any event, a complete comparison of the observable distinctions predicted by the two scenarios still awaits full, three-dimensional radiation transport simulations carried out at high enough resolution to resolve physical processes at very small scales. Into this fray, Wang *et al.* now step, armed with an upstart and potentially powerful observational tool: the ability to study the geometry of the supernova ejecta by analyzing the polarization properties of the light coming from the star shortly after explosion.

Are supernovae round? Simple to pose, this question belies a menacing observational challenge, given that all extragalactic supernovae remain point-like in the night sky throughout the critical early phases of their evolution. Fortunately, geometric information is encoded in the polarization properties of supernova light. The essential idea is that photons become polarized when they scatter off of free electrons, and hot, young supernova atmospheres contain an abundance of free electrons. Indeed, if we could view such an atmosphere as an extended source rather than as an unresolvable point of light, we would expect to measure changes in both the direction and strength of the polarization as a function of position in the atmosphere. For a spherical, unresolved source, the directional polarization components cancel exactly and yield zero net polarization. Any deviation from perfect symmetry or roundness of the source in the plane of the sky, however, gives rise to a net polarization (see the figure).



Probing supernova properties. A spherical, unresolved supernova atmosphere produces zero net polarization (**top**), whereas a nonspherical atmosphere does not (**middle**). Clumps of material that unevenly block the photosphere's light can also produce a net supernova polarization (**bottom**), and it is this mechanism that is thought to be responsible for the majority of the observed polarization of SNe Ia.

There are two basic causes of supernova polarization. One is asphericity of the electron-scattering atmosphere; because electron scattering is independent of wavelength, it generally produces a uniform increase in the overall polarization level across the spec-

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trum. In the other mechanism, asymmetry in the distribution of material ("clumpy ejecta") above the electron-scattering photosphere unevenly screens the underlying light. Unlike global asphericity, this second mechanism is strongly dependent on wavelength, because only those spectral regions corresponding to line transitions of the chemical elements that make up optically thick clumps will be polarized.

From spectropolarimetry gathered on seven events, previous work in this young field has found SNe Ia to have low overall polarizations but occasionally strong line polarization features (*4*–*7*). The emerging picture is thus one of a globally spherical photosphere with clumpy (or otherwise asymmetrically distributed) ejecta overlying it. How can such studies shed light on the type Ia flame-propagation mystery? The latest models indicate that pure deflagrations leave behind lumpier ejecta than delayed detonations do (*3*, *8*).

Spotting trends in SNe Ia data has a long tradition of bearing rich fruit. In 1936, Walter Baade pointed out that the substantial homogeneity and extraordinary brightness of these objects could make them powerful cosmological tools. By the early 1990s, however, it became clear that the dispersion in peak intrinsic luminosity (by more than a factor of 10) complicated their use as "standard candles." The fix came in 1993, when Phillips (*9*) quantified a trend first noticed by Pskovskii (*10*) that intrinsically bright SNe Ia rise and decline in brightness more slowly than dim ones do. Various versions of the "light curve–width" relation have since provided the edifice upon which the entire SN Ia cosmology enterprise has been built, and served as touchstones for theoretical models of the explosions.

It is just such a trend that Wang *et al.* now identify in spectropolarimetry of 17 SNe Ia: Bright events show systematically weaker line polarization than dim ones do. This trend is consistent with the idea that different SNe Ia make the transition from deflagration to detonation at different times. The sooner it happens, the brighter the supernova and the more completely scoured the ejecta will be of the clumps left behind by the deflagration front. The agreement between model predictions and observations strengthens the case for a detonation phase.

Will all debate now end on the subject? It is doubtful. Critics will point out that the trend identified by Wang *et al.* specifically excludes all spectroscopically "peculiar" SNe Ia, which may constitute 30% or more of the total population (*11*). Fundamental advances often come from consideration of the differences seen in a sample, rather than from the similarities alone. And some are likely to withhold any judgment until full three-dimensional models capable of resolving the clumps and quantitatively tracking the resulting polarization become available. Simply put, too many mysteries still surround SNe Ia for anyone to grow complacent. An important clue appears to have been wrested from nature, but we are not ready to resolve the riddle of SNe Ia just yet.

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ANTHROPOLOGY

The Missing Years for Modern Humans

Fossil, archaeological, and DNA evidence of three separate research teams provide insight into the period between the emergence of *Homo sapiens* in Africa and their appearance in Europe.

Ted Goebel

Current interpretations of the human fossil record indicate that fully modern humans emerged in sub-Saharan Africa by 195,000 years ago (*1*). By 35,000 years ago, modern humans thrived at opposite ends of Eurasia, from France to island southeast Asia and even Australia. How they colonized these and other drastically different environments during the intervening 160,000 years is one of the greatest untold stories in the history of humankind. Two reports on pages 226 and 223 of this issue (*2*, *3*) and one in a recent issue of *Science* (*4*) interpret some of the chapters of this story.

To understand the dispersal of modern humans, we must know when these populations expanded from Africa into Eurasia. For the past 20 years, many researchers in this field have been under the impression that this event could have occurred as early as 100,000 years ago (*5*), but new genetic evidence indicates that the spread out of Africa occurred much more recently, closer to 60,000 to 50,000 years ago (*6*).

However, independent corroborating evidence of this recent-dispersal hypothesis is required. Grine *et al.* (*2*) provide a first important test through the analysis of the modern human skull from Hofmeyer, South Africa. This skull was originally discovered in 1952, but it came from an eroded context and not an archaeological excavation and did not yield sufficient collagen for accurate radiocarbon dating. Using a combination of other dating techniques, Grine *et al.* show that sediment within the skull's endocranial cavity was deposited about 36,000 years ago.

Thus, here is the first skull of an adult modern human from sub-Saharan Africa that dates to the critical period, and one that can speak to the relationship of early moderns from Africa and Europe. The Hofmeyer skull is morphometrically more similar to modern humans of Upper Paleolithic Europe than to recent South Africans or Europeans, and it has little in common with Neandertals. Thus, 35,000 years ago, modern populations of sub-Saharan Africa and Europe shared a very recent common ancestor, one that likely expanded from east Africa 60,000 years ago (*7*) (see the figure). This population not only spread south into South Africa but also east into Eurasia, navigating across the Bab el-Mandab Strait of the Red Sea from the Horn of Africa to southern Arabia (*6*).

Archaeological evidence of the hypothesized passage across the Red Sea still eludes us, but the fossil and archaeological records for southeast Asia and Australia indicate that

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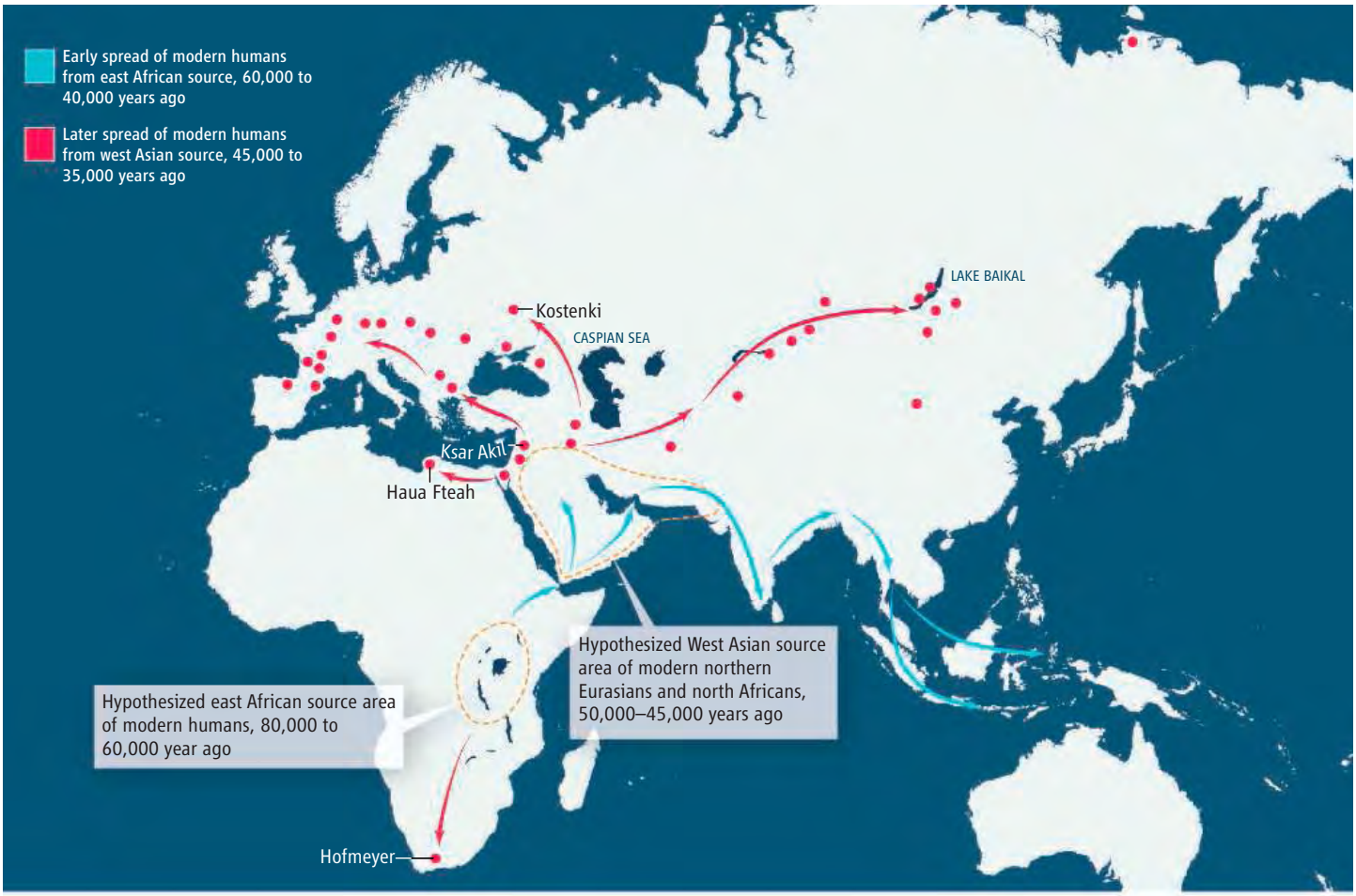

Human pathways. Reconstructed spread of modern humans during the late Pleistocene, and locations of some key early Upper Paleolithic archaeological sites. Grine *et al.*, Olivieri *et al.*, and Anikovich *et al.* provide new evidence confirming that early modern humans spread from southwestern Asia into northern Africa, Europe, and Russia about 45,000 to 40,000 years ago.

moderns had arrived in these regions by 50,000 years ago (*8*). The road east likely followed the south Asian coastal margin, a route requiring few modifications in adaptation other than those mandated by the initial exodus from Africa.

The spread north, however, required more time for adaptation to cope with colder temperatures, drier climates, and—most challenging of all—Neandertals. Despite these constraints, genetic records suggest that sets of genes, called haplotypes, carried by the first moderns into northern Eurasia existed by 45,000 years ago. Precisely where they evolved remains unknown; possibilities include southern Arabia, India, or other regions of interior western Asia (*6*, *9*). In any case, the outcome was a series of concomitant founding migrations about 40,000 years ago from western Asia to the Mediterranean, temperate Europe, Russia, and central Asia.

The best-known of these migrations is the move northwest into temperate Europe by modern humans (*10*, *11*), which led to Neandertal extinction after a short period of interaction (*12*–*15*). The other expansions out of western Asia presumed by the genetic evidence are not well understood. The reports by Olivieri *et al*. (*4*) and Anikovich *et al*. (*3*) provide important clues about them.

Olivieri *et al.* focus on mitochondrial DNA as a tool for researching modern human dispersal from western Asia. Their analysis suggests that two genetic lineages, the M1 and U6 haplogroups, originated simultaneously in western Asia between 45,000 and 40,000 years ago and from there spread with modern humans westward into northern Africa. The estimated timing of this event should not come as a surprise to archaeologists who interpret similarities in tool technologies and artifact forms as indicators of prehistoric population relationships. Through this "technocomplex" approach, they for years have theorized a historical link between the first Upper Paleolithic stone blade technologies in the Levant (called "Aurignacian" at sites like Ksar Akil, Lebanon) and similar blade technologies in northern Africa (called "Dabban" at sites like Haua Fteah, Libya) (*16*). Together the genetic and archaeological records indicate that the modern humans spread from the Levant into Mediterranean Africa by 40,000 years ago (*13*, *14*).

Another intriguing scene in the emerging story of modern humans is being played out at the famous Kostenki sites along the Don River, Russia, about 500 km south of Moscow. There, Anikovich, Sinitsyn, Hoffecker, and colleagues have unearthed archaeological evidence that the Upper Paleolithic—characterized by a series of new technologies and behaviors that are decidedly modern—had begun by 45,000 years ago. Because of perceived problems with the radiocarbon record of this time, they use optically stimulated luminescence techniques and precise chronostratigraphic correlations to define the age of the archaeological assemblages in question. The assemblages contain not just stone blades typical of the early Upper Paleolithic elsewhere in western Eurasia, but also some unique bone and ivory tools, perforated shell beads, and a carved chunk of ivory that may represent the head of an unfinished human figurine.

Although the early Kostenki assemblages are based on blade tools like those in other early Upper Paleolithic technocomplexes in Europe, this is where the similarities end and differ-

ences begin. First, the early Kostenki assemblages lack diagnostic artifacts of the Aurignacian, for example, split-based bone points, carinated end scrapers, and strangled blades. Second, they contain tool forms that are rare or absent in the typical Aurignacian, including dihedral burins, bifacial knives, and perforated fossil ornaments. As Anikovich *et al*. explain, the early Kostenki technocomplex is not Aurignacian; nor is it "transitional," reflecting a local shift from the Middle to Upper Paleolithic. Instead, it is something new and different: a fully developed Upper Paleolithic technocomplex with no European analog and no obvious root in the local Russian Middle Paleolithic. Although only human teeth not identifiable to species have been found associated with these early Kostenki assemblages, Anikovich *et al.* argue that they represent a pioneering group of moderns. If this is true, then the implications are clear: The first moderns to colonize European Russia may not have spread from the Levant via central Europe, but instead from interior western Asia via the Caucasus Mountains or from further east central Asia. This point of origin is consistent with the prediction by Olivieri *et al.* that modern Europeans developed out of several "regional enclaves" in greater western Asia.

So what we infer is this: Modern humans spread out of Africa very late in the Pleistocene—as recently as 60,000 to 50,000 years ago. One founding population spread east, reaching Australia by 50,000 to 45,000 years ago. Another remained in southwestern Asia or India, but after ~5000 to 10,000 years, its descendant populations dramatically expanded their range, colonizing lands as far removed from one another as northern Africa, temperate Europe, and the Russian Plain. They also reached southern Siberia by 45,000 years ago (*17*) and arctic Siberia by 30,000 years ago (*18*), but the retelling of these and other events in the missing years of modern human evolution must await new fossil and archaeological discoveries as well as continued DNA sampling of the world's living populations.

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PHYSICS

Electron Nematic Phase in a Transition Metal Oxide

Materials in which electrons interact strongly can exhibit unusual properties. Electrons have now been observed to assemble into a pattern like that seen in liquid crystals.

Eduardo Fradkin, Steven A. Kivelson, Vadim Oganesyan

Each time we encounter a new quantum state of matter, we learn more about the possible behavior of condensed matter systems. On page 214 of this issue, Borzi *et al*. (*1*) report their observation of an unusual metallic phase in ultrapure strontium ruthenate ($Sr_3Ru_2O_7$). Discovery of this state, known as a nematic phase, confirms earlier theoretical predictions and shows that electrons can assemble into some very exotic structures.

$Sr_3Ru_2O_7$ is a layered crystal with "tetragonal" symmetry, which means that it looks the same when rotated by 90°. Consequently, the electrical resistance should be the same in the x or y direction (if z is the rotation axis). Borzi *et al*. found that, for a limited range of magnetic fields and for temperatures below a critical temperature, this symmetry is spontaneously broken. That is, the resistivity in one direction becomes larger than the other by as much as a factor of 2. Spontaneous symmetry breaking is one of the defining features of phases of matter; for instance, although water is a uniform liquid, when cooled below its freezing temperature, it forms ice crystals with facets and even snowflakes with all sorts of beautiful spatial structures, which reflect the fact that at a molecular level, the atoms have spontaneously arranged themselves into a crystalline lattice. The symmetry breaking observed by Borzi *et al*. confirms the existence of the nematic phase.

In the conventional description of metals, the strongly interacting conduction electrons can be accurately represented as a gas of weakly interacting electron-like excitations, referred to as "quasiparticles." This description, known as Fermi liquid theory, works for many metallic systems. Under the right conditions, a Fermi liquid will transform into a low-temperature superconductor (in accord with the Bardeen-Cooper-Schrieffer theory) or, in some cases, into a charge- or spin-density wave. However, over the past two decades, new types of metallic materials with strongly correlated electrons have been discovered that do not fit this standard description. The list now includes the superconducting copper oxides, the colossal magnetoresistive manganites, and many other materials. Although Fermi liquid theory can account for some properties of these highly correlated materials, for broad ranges of temperature and composition this description fails utterly. Moreover, because of the strong interactions, a far broader spectrum of broken-symmetry phases might be possible. Indeed, several new quantum phases of matter have been discovered in the past 20 years, including "*d*-wave" superconductivity in the high-temperature superconductors and time-reversal symmetry-breaking superconductivity in Sr_2RuO_4 (a close relative of $Sr_3Ru_2O_7$) (*2*). The nematic metallic phase of $Sr_3Ru_2O_7$ discovered by Borzi *et al*. expands the list of new phases.

Fermi liquid behavior occurs where the Fermi energy (the quantum kinetic energy) of the electron fluid dominates the particle dynamics. In contrast, where the Coulomb interactions are dominant, electrons are known to form an insulating electron crystal. The electron fluids in strongly correlated metals lie in an intermediate range where neither of these energies is dominant. An analogy can be made with complex classical fluids, as shown in the top panel of the figure. There is generally a gas phase at high temperatures, where entropy

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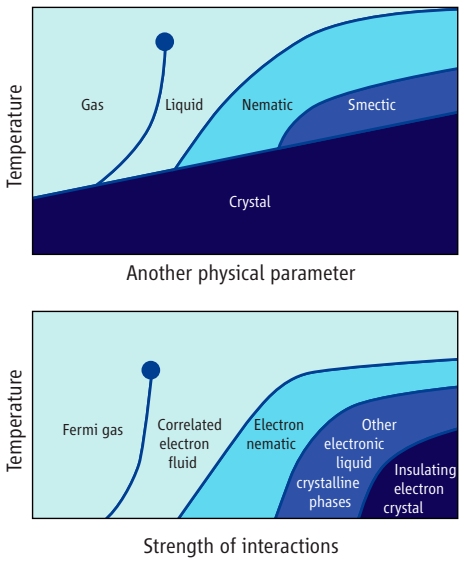
dominates, and a crystalline solid phase at low temperatures, where interaction energies dominate. Sometimes this is all there is; the solid sublimes at a critical temperature and there are no intermediate highly correlated classical fluid phases. However, in many cases there is an intermediate liquid phase in which interactions and entropic considerations must be treated on an equal footing. In some cases, there are additional "liquid crystalline" phases (e.g., nematic and smectic) that can flow like a liquid but exhibit patterns of broken symmetry that are somewhat like those of a solid. In 1998, two of us in collaboration with Emery (*3*) argued that strong correlations can induce electronic liquid crystal phases. These phases are quantum fluid (conducting) states with a pattern of spontaneous symmetry breaking that is intermediate between those of the simple fluid and an electron crystal (see the bottom panel of the figure).

The nematic metal is named in analogy with a classical uniaxial nematic liquid crystal. The classical nematic is typically visualized as a liquid of cigar-shaped molecules in which the positions of the molecules fluctuate randomly in space but the long axes of the molecules are preferentially aligned in one direction. It may thus seem strange that a fluid of point particles—electrons—can form a nematic state. However, one can think of an electron nematic as a partially (quantum) melted version of an anisotropic electron crystal. Under appropriate circumstances, correlated materials exhibit "stripe order" in which the electrons form a striped pattern that breaks translational symmetry and chooses a preferred axis in the crystal. One can imagine a nearby phase in which quantum fluctuations melt the stripe order but the preferred direction of the stripes remains as a memory of the proximate ordered phase. In this view, little fragments of stripes play the role of the cigar-shaped molecules of classical nematics.

The theory of a partially melted crystal is still being developed. An alternative approach to the metallic nematic phase considers the effect of increasingly strong interactions in Fermi liquids. It has long been known that a Fermi liquid becomes thermodynamically unstable if certain interactions are sufficiently strongly attractive. It was shown (*4*, *5*) that in some cases, rotational invariance is spontaneously broken while translation symmetry is preserved. Subsequent work, which focused on the important role of the lattice, showed that this is an Ising nematic phase with a quantum phase transition, usually first order, between two phases with different Fermi surface topologies (*6*, *7*), while the thermal transition remains second order. Nevertheless, it



Electron ordering. (**Top**) Schematic phase diagram of a classical complex fluid. The physical parameter along the horizontal axis could be pressure, composition, or the like. (**Bottom**) Schematic phase diagram of the electron fluid in a strongly correlated metal. The quantity along the horizontal axis measures the strength of the interactions relative to the Fermi energy; it could be, for example, the inverse of the electron density or the strength of the electron-phonon coupling. Although the same phase names are used, in the case of classical fluids, the relevant symmetries are the continuous translational and rotational symmetries of free space, whereas in the electronic case, the relevant symmetries are the discrete crystal symmetries of the host material.

was found (*8*) that whenever a quantum critical point is accessible, the Ising nematic has the same non-Fermi liquid behavior predicted by the continuum theory (*4*).

As mentioned by Borzi *et al.*, although the nematic metal they discovered is a first, there is precedent: Quantum Hall nematic phases occur below 150 mK in gallium arsenide heterostructures (*9*). In contrast to the nematic metal discovered by Borzi *et al.*, the quantum Hall nematic only occurs in two dimensions, and crystal field effects of the host lattice are largely negligible. Moreover, in the quantum Hall case, the magnetic energy scale (i.e., the cyclotron energy, $\hbar\omega_c$) is dominant, whereas in $Sr_3Ru_2O_7$, the Fermi energy is 10^3 times as large as the cyclotron energy. Highly suggestive evidence of a nematic phase has also been reported previously by Ando *et al.* (*10*) in underdoped samples of the high-temperature superconductor $YBa_2Cu_3O_{6+y}$. The difficulty is that $YBa_2Cu_3O_{6+y}$ is itself orthorhombic, so the crystal structure already chooses a preferred direction. To infer the existence of an electron nematic, Ando *et al.* argued that the measured resistance anisotropy is too large and too temperature-dependent to be a consequence merely of the crystalline orthorhombicity and that, moreover, as a function of oxygen concentration, y, the anisotropy increases as the orthorhombicity decreases (*11*).

The observation of a nematic phase in $Sr_3Ru_2O_7$ strikingly demonstrates the existence of electronic liquid crystal phases in strongly correlated systems. Why have they not been identified before? Disorder has a devastating effect on nematic phases. Impurities create local electric fields whose gradients couple directly to the nematic order parameter. In two dimensions, even the weakest disorder is sufficient to destroy the long-range nematic order, which explains why macroscopic anisotropy is seen only in the cleanest systems. A second problem, illustrated in the work of Ando *et al.*, is that there is always a coupling between the electron fluid and the crystalline lattice. If the electron fluid spontaneously chooses a preferred axis, this will cause a lattice distortion of similar symmetry and, conversely, a lattice orthorhombicity will always induce anisotropy in the electronic properties. It can be difficult to tell which is the chicken and which the egg. In $Sr_3Ru_2O_7$, any symmetry-breaking (orthorhombic) lattice distortion is so small that it is currently unmeasurable, so the case for an electronic nematic is unambiguous. In light of the work of Borzi *et al.*, the case made by Ando *et al.* for the existence of a nematic phase in the high-temperature superconductors is considerably strengthened and, more generally, the door is opened for interpreting puzzling data in a variety of other strongly correlated materials in terms of electronic liquid crystalline phases.

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MICROBIOLOGY

A Proteomic Snapshot of Life at a Vent

Survival of a polychaete worm in a deep sea hydrothermal vent depends on complex metabolic interactions with symbiotic bacteria.

Charles R. Fisher and Peter Girguis

In the late 1970s, scientists discovered rich communities of animals living around deep-sea hydrothermal vents in the eastern Pacific. Since then, hundreds of other chemoautotrophic-based communities and animals have been discovered. What were once thought of as biological oddities are now confirmed to be abundant in all the world's oceans. What the sites all have in common is an abundance of reduced chemicals (such as sulfide or methane) at the sea floor. Many of the sites, including mineral-rich hydrothermal vents and the oil- and gas-rich hydrocarbon seeps, are increasingly affected by anthropogenic activities. To assess the effects of these activities, we must better understand the lifestyles of the creatures that thrive in this extreme habitat. The use of powerful molecular tools, such as the approach described by Markert *et al*. on page 247 of this issue, will advance our understanding of the abundant chemoautotrophic symbioses of the deep sea.

One of the most prominent members of the hydrothermal vent community is the tubeworm *Riftia pachyptila*, a siboglinid polychaete that has become the unofficial poster child for hydrothermal vents. *Riftia* has no mouth, gut, or anus and cannot feed by normal means. Instead, *Riftia* depends on intracellular chemoautotrophic symbionts—which fill a large internal organ called the trophosome—for nutrition. The symbionts are γ-proteobacteria, which are functionally analogous to plant chloroplasts in that they generate organic carbon as a food source for their worm host (using sulfide as an electron donor and oxygen as an electron acceptor). Key to understanding the biology of *Riftia* and other chemoautotrophic symbioses is an understanding of the biology of their symbionts. However, no chemoautotrophic symbiont has ever been cultured in a laboratory, and this has long hampered our ability to study their metabolism. Markert *et al.* use a proteomic approach to examine protein expression in *Riftia* symbionts and gain new insights into their biochemistry and metabolism (*1*).

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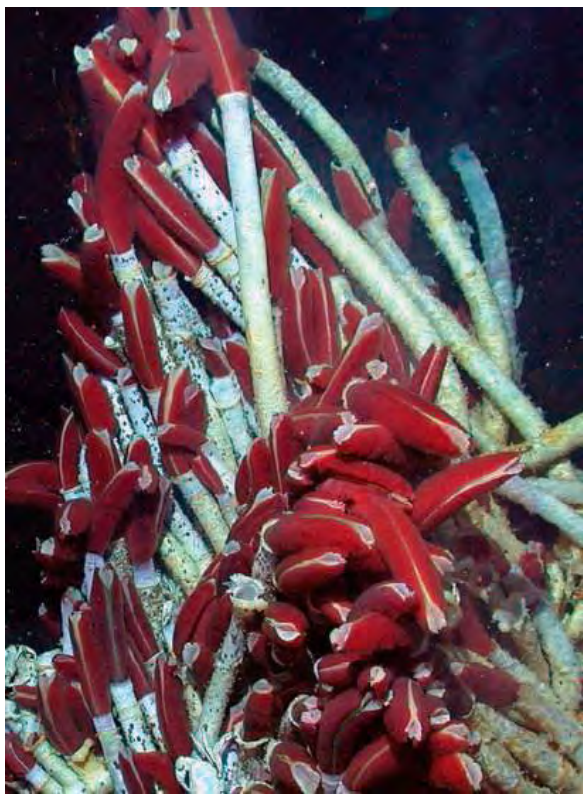
Hydrothermal vents are dynamic and potentially dangerous environments. Temperatures can range from near freezing to more than 300°C over centimeters. Within animal communities, temperatures can vary over more than 40°C within seconds. Hydrothermal vents are also quite ephemeral, with local sources of hydrothermal flow often lasting only a few years. Consequently, the habitat fluctuates, and vent fauna must balance exposure to the hot and potentially toxic vent fluid with the need to obtain nutrition either directly from the fluid or from microbes living in the fluid. *Riftia* is supremely adapted for its symbiotic life style in this environment. They live with their highly vascularized gill-like plumes exposed to vent fluid and have circulating hemoglobins that bind to both oxygen and sulfide reversibly and with high affinity and capacity (*2*, *3*). This allows *Riftia* to take up and store large amounts of these chemoautotrophic substrates, transport them through its tissues with no harmful effects, and provide its symbionts with a bountiful supply of both (*4*). In return, the symbionts are extremely efficient and productive, fixing carbon at high rates to support the host's growth (*5*). This suite of adaptations enables *Riftia* to be very fast growing and quite fecund, while reliant on its symbionts for nutrition.

These and earlier studies of *Riftia* focused on characterizing its major biochemical, physiological, and ecological attributes, such as hemoglobin properties, oxygen uptake rates, and habitat characteristics. More recent studies have grown in both breadth and depth, investigating the expression of genes, quantifying the metabolic interactions between host and symbiont, and describing the ecological dynamics of *Riftia* aggregations. Markert *et al.* have now used the power of genomic analyses coupled with high-throughput protein profiling to obtain a snapshot of the proteins (or proteome) expressed by the *Riftia* symbiont. Their results illustrate the degree to which *Riftia* symbionts are poised for high rates of chemoautotrophic carbon fixation powered by sulfide oxidation. For example, Markert *et al*. find that 12% of the total cytosolic proteome of these symbionts consists of three proteins involved in coupling energy production to sulfide oxidation. This is a marked departure from fast-growing, free-living heterotrophic bacteria that instead expend a considerable fraction of their energy synthesizing amino acids for their own cell division and growth (*6*). The prominence of these three proteins underscores the central role of the symbionts: to provide nutrition to the association by harnessing energy from sulfide.

The mechanism of inorganic carbon uptake and transport by the host, and fixation by the symbiont, has been the subject of much inquiry and debate. The first indication that hydrothermal vent fauna obtain their nutrition from chemoautotrophic sources (not from photosynthetically derived products but from primary production powered by hydrogen



The life aquatic. *Riftia pachyptila* tubeworms at a hydrothermal vent field located near 21°N on the East Pacific Rise.

CREDIT: B. VRIJENHOEK/MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

sulfide) came from analyses of their stable carbon (^{13}C) and nitrogen (^{15}N) isotope contents (*7*, *8*). The amount of these isotopes detected in dominant fauna (tube worms, mussels, and clams) did not reflect normal deep-sea carbon and nitrogen. Later studies demonstrated the presence of intracellular chemoautotrophic symbionts in these animals, confirming the nonphotosynthetic nutritional source of carbon and nitrogen. Mussels and clams had δ^3C values of about –30 per mil (‰), in the range that was expected for carbon that is derived from chemoautotrophic bacteria. However, the $\delta^{13}C$ value of *Riftia* was much higher (~ –15‰) and consequently more difficult to understand. A variety of explanations have been put forward to explain these isotope values, but none has proven completely satisfactory (*9*–*11*).

Markert *et al*. find high amounts of enzymes involved in the reductive tricarboxylic acid cycle in extracts of the *Riftia* symbiont and suggest that this is an important pathway of carbon fixation by the symbiont. In addition to the implications for more energy-efficient carbon fixation, this finding may help explain the anomalously high carbon isotope values that have puzzled researchers for decades.

Far less than 1% of the microbes present in nature have been successfully cultured in the laboratory. No chemoautotrophic symbiont has yet been cultured, and it is possible that many never will be. Not only is the milieu of a living host difficult to imitate in vitro, but in some cases, the exchange and integration of host and symbiont genes may have yielded a symbiont more analogous to an organelle than to a free-living microbe. In such instances, genomic and proteomic approaches provide valuable information on the symbiont's metabolic capabilities and evolutionary history. Quantitative proteomics has the additional value of allowing one to use protein expression levels as a metric for studying the importance of metabolic pathways used by these symbiotic microbes in situ.

Many questions remain about these enigmatic animals and their rather extreme life styles. *Riftia*'s trophosome, which is packed with billions of bacteria per gram of tissue, is intertwined with the animal's gonads. Considering the rarity of active hydrothermal vents on the sea floor, and the improbability of larvae finding a suitable home, it is likely that a high percentage of the nutritional input from the symbionts goes directly to reproduction. How is this accomplished and coordinated? Furthermore, transmission of the symbionts between generations is not direct because the larvae are aposymbiotic and newly settled tube worms must acquire their symbionts anew each generation from an apparently free-living pool. How is the metabolism of the free-living stage different from that of the symbiotic stage? Once contact with a host is made, how do the symbionts contribute to successful establishment of the symbiosis? Molecular approaches like that of Markert *et al*. may help answer such questions about life and relationships in this remote and inhospitable environment.

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MOLECULAR BIOLOGY

Amplified Silencing

Small RNA molecules that silence gene expression are amplified by different mechanisms in nematodes and plants.

David C. Baulcombe

Ten years ago, we knew nothing about how double-stranded RNA blocks gene expression through the silencing of targeted RNA. We now have a good understanding of this process, and current interest is turning to variations on the basic mechanism. Recent studies involving plants and the nematode *Caenorhabditis elegans* continue this trend, including those reported in this issue by Pak and Fire on page 241 (*1*) and Sijen *et al.* on page 244 (*2*). Two other papers by Axtell *et al.* (*3*) and Ruby *et al.* (*4*) are also relevant. These studies deal with the amplification of silencing-related RNA and explain how strong, persistent silencing can be initiated with small amounts of "initiator" double-stranded RNA. The amplification process has implications for application of RNA interference to control gene expression in biotechnology and for understanding the effects of silencing RNAs on cell function and organism development.

Specifically, these new studies investigate how the target of silencing can spread (or transit) within a single strand of RNA. The initiator of transitivity is a double-stranded RNA that is first processed by Dicer, a ribonuclease III–like enzyme, into short interfering RNA (siRNA) or a related type of RNA referred to as microRNA (miRNA). These 21- to 25-nucleotide single-stranded RNAs are the primary silencing RNAs in the transitive process. A primary silencing RNA binds to a ribonuclease H–like protein of the Argonaute class. The resulting Argonaute ribonucleoprotein can target long RNA molecules by Watson-Crick base pairing. The targeted RNA then becomes a source of secondary siRNAs. Transitivity occurs when the secondary siRNAs correspond to regions adjacent to the target sites of the primary silencing RNA.

RNA-directed RNA polymerases (RdRPs) produce secondary siRNA, and the new results indicate that they catalyze two different mechanisms of silencing amplification. One mechanism is characterized by Axtell *et al*. (*3*), who investigated endogenous secondary siRNAs in plants. They show that efficient secondary siRNA production occurs if a single-stranded RNA has two target sites for the Argonaute ribonucleoprotein. Optimal secondary siRNA production occurs when the targeted RNA is cleaved by Argonaute. Cleaved RNA then recruits RdRP, which generates double-stranded RNA. Dicer then produces transitive secondary siRNAs (see the figure).

Another biogenesis mechanism of secondary siRNAs has, so far, only been described in *C. elegans*. The discovery of this distinct mechanism by Sijen *et al*., Pak and Fire, and Ruby *et al*. follows from the observation that a type of siRNA is underrepresented in

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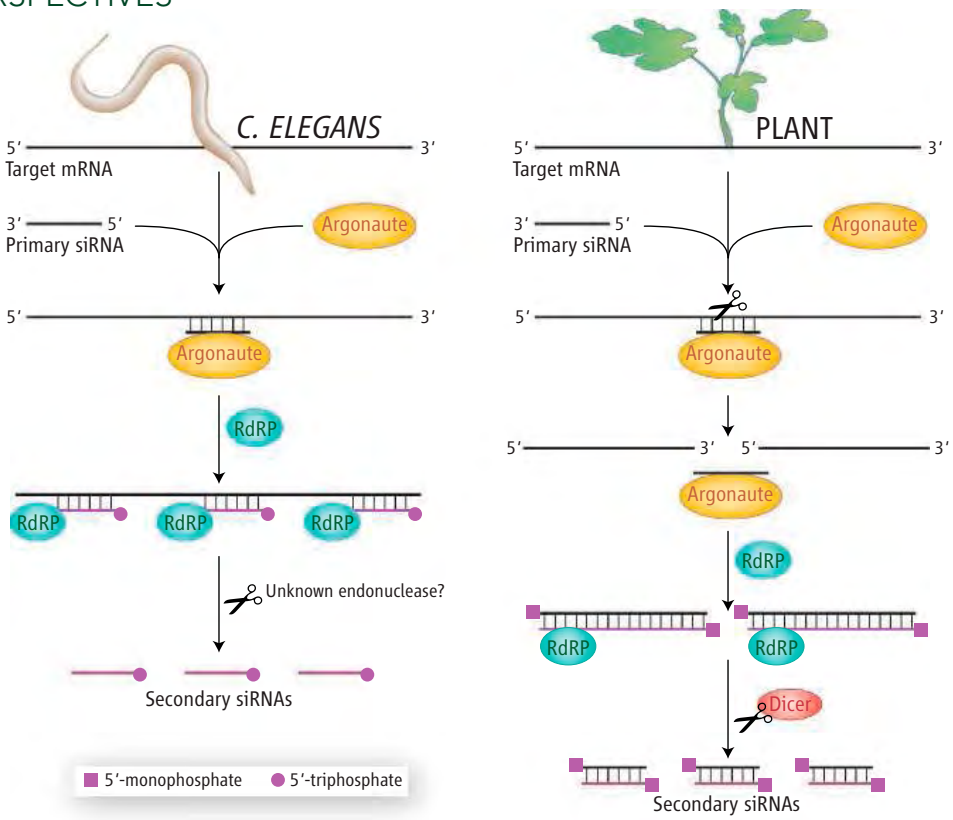

Secondary siRNA production in plants and animals. Secondary siRNAs are produced by RdRP-mediated transcription of RNA that has been targeted by a primary siRNA or miRNA. In *C. elegans* (**left**), an Argonaute protein associated with a primary siRNA targets a long single-stranded RNA and recruits an RdRP that synthesizes 22–23 nucleotide secondary siRNAs directly. In plants (**right**), the recruitment of RdRP is optimal when the long single-stranded RNA has two targets for primary siRNA or miRNA (only one is shown). The targeted RNA is then converted to long double-stranded RNA by the RdRP and secondary siRNAs are generated after cleavage by Dicer.

sequence databases. This scarceness is because these siRNAs have a 5′-triphosphate and are thus excluded by the standard methods for cloning and sequence analysis. These methods are normally specific for RNA with a 5′-monophosphate, the hallmark of Dicer cleavage.

In addition to their 5′-triphosphorylation, these siRNAs are distinct from the primary silencing RNAs in that they have a strand bias. They predominantly are antisense to the target of the primary silencing RNA. The secondary siRNAs also have the surprising characteristic that they are phased relative to each other (*2*, *4*): The first siRNA covers 22 nucleotides starting close to the target site of the primary siRNA, the second siRNA is then the adjacent 22 nucleotides, and so on (see the figure). One explaination for these features might be that the 5′-triphosphorylated secondary siRNAs are generated when RdRPs are recruited to a target of the primary silencing RNA. Short antisense RNAs are then synthesized de novo, and the presence of the 5′-triphosphate in the first incorporated nucleotide is diagnostic of secondary siRNAs made by this mechanism. Sijen *et al*. rule out primary siRNA as a primer in this mechanism because mismatches in its sequence relative to that of a target RNA are absent in the secondary siRNAs. To explain the rather precise size (22 or 23 nucleotides) of the secondary sRNAs, this model requires that the RdRP automatically terminates RNA synthesis at a defined site or that the transcription products be cleaved at their 3′ end by an unidentified endonuclease.

What is the natural role of these transitive secondary siRNAs? In plants, they target messenger RNAs (mRNAs) (*3*), and it is likely that they do the same in *C. elegans* because endogenous siRNAs with 5′-triphosphate correspond to the antisense of mRNA coding sequences (*1*). Moreover, Yigit *et al*. (*5*) describe how secondary siRNAs are bound to a specific class of Argonaute proteins and that they direct RNA cleavage. It is likely, therefore, that secondary siRNAs regulate gene expression in situations where amplification of silencing is important.

A clue to the type of situation in which secondary siRNA might be important comes from experimental RNA interference in *C. elegans* and transitive transgene silencing in plants. In both systems, transitivity and secondary siRNA production amplify silencing-related RNAs so that silencing persists in the absence of the initiator double-stranded RNA. In some instances associated with this persistence, there are epigenetic effects at the DNA or chromatin level (*6*, *7*). On the basis of these observations, and reasoning that experimental systems may illustrate elements of the natural mechanisms, it seems likely that the endogenous secondary siRNAs could mediate effects of silencing that persist in the absence of the initiator double-stranded RNA. Perhaps the amplified secondary siRNAs influence processes such as developmental timing in which the effects of a silencing trigger might persist after their initial induction. Consistent with this idea, secondary siRNAs in the plant *Arabidopsis thaliana* affect the timing of the developmental transition between adult and juvenile growth phases (*8*).

In addition to the biological implications of the amplification mechanisms, there are two technical issues. First, from a biotechnological perspective, it would be advantageous if the amplification mechanisms could be harnessed to enhance silencing in therapeutic or genomic applications. The absence of RdRP genes in the fly *Drosophila melanogaster* and in mammalian genomes indicates that this effect might not be possible in all organisms. However, there are recently described siRNA-like species in *Drosophila* (*9*) with the phased and strand-bias characteristics of secondary siRNAs in *C. elegans*. Perhaps there are other enzymes in mammals that can substitute for the RdRP proteins in an amplification process. The second technical point is a cautionary message about methods for high-throughput sequencing of siRNA populations. Secondary siRNAs with 5′-triphosphates are excluded from many of the methods associated with this technology, and amplified siRNAs would be missed. Fortunately, two of the *C. elegans* papers (*1*, *2*) describe methods for cloning and sequencing siRNA with 5′-triphosphate. We will now see to what extent the existing sequence databases will need to be revised to account for 5′-triphosphorylated siRNAs.

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Proteasome-Independent Functions of Ubiquitin in Endocytosis and Signaling

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Ubiquitination is a reversible posttranslational modification of cellular proteins, in which a 76–amino acid polypeptide, ubiquitin, is primarily attached to the ε-amino group of lysines in target proteins. Ubiquitination is a major player in regulating a broad host of cellular processes, including cell division, differentiation, signal transduction, protein trafficking, and quality control. Aberrations in the ubiquitination system are implicated in pathogenesis of some diseases, certain malignancies, neurodegenerative disorders, and pathologies of the inflammatory immune response. Here, we discuss the proteasome-independent roles of ubiquitination in signaling and endocytosis.

Ubiquitin (Ub) is a highly conserved protein of 8 kilodaltons that becomes covalently attached to lysine (Lys) residues of target proteins. Protein-attached Ub is a substrate for the attachment of further Ub residues, which leads to the formation of a polyubiquitin chain. Classically, polyubiquitination is a signal that directs proteins to the proteasome, where the Ub is recycled and the protein is degraded (*1*). Ubiquitination also remodels the surface of substrate proteins and thereby potentially affects properties such as stability and activity (*2*), drives interactions with other proteins, and plays roles in subcellular localization. Diverse forms of Ub modifications exist: Monoubiquitination is the attachment of a single Ub to a protein; multiubiquitination occurs when several Lys residues of the target protein are tagged with single Ub molecules; and polyubiquitination denotes the addition of a Ub chain made of several ubiquitins that are linked through the C-terminal glycine residue of each Ub unit and a specific internal Lys of the previously attached Ub. Monoubiquitination or multiubiquitination has been shown to be required for the entry of certain cargo proteins into vesicles at different stages of the secretory/endocytic pathway (Fig. 1) (*3*), whereas polyubiquitination has been mainly associated with proteasomal degradation, although it certainly has a broader function (*4*). In the case of polyubiquitination, there can be at least seven different linkages between ubiquitins, because there are seven internal lysines in Ub. The role of different linkages in polyubiquitins has begun to be elucidated in recent years. Linkage through Lys^{48} (Ub^{K48}) is mainly used for targeting to the proteasome, and Lys^{63} (Ub^{K63}) linkages seem to play important roles in DNA damage tolerance, inflammatory response, the endocytic pathway, and ribosomal protein synthesis (*4*). Ub can also be removed from proteins, and different Ub hydrolases that regenerate free Ub have been identified and implicated in regulation of various cellular events (*5*–*7*). Protein modules containing Ub-binding domains (UBDs) have also been identified and are being characterized. The identification of many UBDs with diverse structural folds, by which specific Ub modifications can be recognized, has helped in understanding the role of ubiquitination (*8*). The presence of several structurally distinct Ub modifications, the exquisite specificity of Ub conjugation, and the existence of UBDs, in which the different Ub modifications are capable of being distinguished, make Ub a multifunctional signal.

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Ubiquitination as an Endocytic Signal

One of the first nonproteasomal functions of ubiquitination was its implication in the process of endocytosis in yeast (*9*, *10*), where monoubiquitination is sufficient as an endocytic internalization signal (*11*) and Ub^{K63} branches facilitate endocytosis (*12*). Many plasma membrane proteins depend on their ubiquitination for internalization in yeast (*13*). On the other hand, the situation is much less clear in animal cells. Early evidence clearly demonstrated a role for the process of ubiquitination for internalization of growth hormone receptor, but ubiquitination of the receptor itself was not required (*14*). Studies on the transforming growth factor–β (TGF-β) receptor suggest that receptor ubiquitination may determine the endocytic internalization pathway that is used. When the TGF-β receptor associated with the Smad7-Smurf2 E3 Ub ligase (*15*), it localized to caveolae and was rapidly degraded after internalization. On the other hand, when the TGF-β receptor associated with Smad anchor for receptor activation, it was internalized via clathrin-coated pits where signaling proceeds from an endosomal compartment (*16*). Therefore, modulation of the endocytic internalization route by Ub could play a critical role in deciding between signal transduction or receptor down-regulation.

Receptor ubiquitination in endocytic internalization is most studied for its potential role in the down-regulation of activated mammalian receptor tyrosine kinases (*17*, *18*), although the situation is still not entirely clear. When epidermal growth factor receptor (EGFR) was stimulated at low epidermal growth factor (EGF) concentrations in HeLa cells, EGFR ubiquitination was not detected, and the receptor localized with clathrin; however, at high EGF concentrations, EGFR was ubiquitinated, and the receptor localized with both caveolae and clathrin. It is not clear that caveolae were sites of internalization in this study. Moreover, with the use of a chimeric protein, EGFR-Ub-mut, that could not be extended by polyubiquitination, a single Ub was sufficient to drive internalization (*19*) in a clathrin-independent manner. Three proteins with UBDs—Eps15, Eps15R, and epsin—that normally localize to clathrin-coated pits were required. In another study, epsin, which is considered to be an endocytic adaptor between ubiquitinated cargo and clathrin, was not able to interact with ubiquitinated cargo in the presence of clathrin (*20*). These results invoked a working hypothesis in which ubiquitination directs clathrin-independent endocytosis and where epsin and Eps15 might be important for coupling ubiquitinated cargo to clathrin-independent internalization. However, in porcine aortic endothelial cells, EGFR is normally polyubiquitinated (Ub^{K63}) before endocytosis on multiple lysines in the kinase domain under low EGF conditions, which should lead to internalization through clathrin-coated pits (Fig. 1). Mutation of the target lysines preventing ubiquitination abrogated down-regulation but not receptor internalization (*21*). Thus, a single Ub can act as an internalization signal in animal cells, but receptor ubiquitination is probably not required for clathrin-mediated internalization of EGFR.

The idea that a single Ub can act as an autonomous endocytic signal is difficult to reconcile with the relative low affinity of UBDs (*8*). However, the low-affinity interactions between monoubiquitin and UBDs could be physiologically relevant in the protein networks formed that drive vesicle budding at the plasma membrane. There are many possibilities for the formation of multiple interactions. Most UBD-containing proteins have additional modules that recognize other proteins or specific phospholipids. For example, epsins have a domain that recognizes phosphoinositides in addition to their three UBDs (*22*). Dual binding to phosphoinositides, generated at specific sites on the membrane, and Ub should allow epsins to stably recognize a monoubiquitin at the plasma membrane in the presence of a pool of cytosolic

Ub. Another possibility is that many Ub-binding proteins have multiple UBDs, and the addition of rather low binding affinities can lead to a cooperative binding reaction and higher avidity. In this case, multiubiquitinated or multimeric cargo molecules would probably be a better substrate. It is possible that the successful demonstration of single ubiquitins working as endocytic signals is related to the fact that both EGFR and the alpha factor receptor are internalized as dimers. Thus, although monoubiquitination may sometimes be sufficient for endocytic internalization, generally productive recognition and uptake of ubiquitinated cargoes requires stabilization of cargo-adaptor complexes through multivalent Ub-UBD interactions. One can speculate how Ub^{K63} polyubiquitination might be particularly adapted to act as an efficient endocytic signal. The extended conformation of Ub^{K63} chains has a linear topology, lining up adjacent Ub chains and making their hydrophobic surfaces available for binding, which should increase the avidity of binding to UBDs (*23*). The higher avidity can thus promote productive internalization of Ub^{K63}-conjugated proteins. Especially for those receptors that have a limited number of cytoplasmic Lys residues and thus cannot be multiubiquitinated, Ub^{K63} polyubiquitination offers an alternate mechanism.

Endocytosis of certain membrane proteins seems to require polyubiquitination. For example, in the case of the β_2-adrenergic receptor (β_2AR), a mammalian heterotrimeric guanine nucleotide–binding protein–coupled receptor, agonist stimulation leads to rapid polyubiquitination of both the receptor and the receptor regulatory protein β-arrestin. It is probably the polyubiquitination of β-arrestin that is necessary for β_2AR internalization (*24*). The yeast protein general amino acid permease (Gap1p) requires the formation of short Ub^{K63} chains for efficient internalization in response to changes in nitrogen status in the medium (*25*). Similarly, Ub^{K63} polyubiquitination of the nerve growth factor (NGF) receptor tyrosine receptor kinase A (TrkA) is required for TrkA internalization and signaling (*26*). It could be that the role of Ub chain topology in vivo is currently underestimated, with Ub^{K63} chains being more important than previously thought. The presence of Ub^{K63} chains may also allow selective recognition by particular UBDs.

Ub and Sorting into Multivesicular Bodies

Ubiquitination also functions as a sorting signal at endosomes targeting its substrates to the interior of the multivesicular body (MVB) (*27*), which is the first step leading to their transport to lysosomes. This pathway is used not only in plasma membrane–receptor down-regulation but also in lysosome biogenesis (Fig. 1). Most proteins that are not ubiquitinated follow other pathways, and those proteins are recycled from endosomes to other compartments. Monoubiquitination or multiubiquitination is critical for sorting of membrane proteins to the interior of the multivesicular endosome in a process that depends on endosomal sorting complex required for transport (ESCRT) complexes, which is detailed in an excellent review (*28*). Ub on receptors or cargo is recognized by the vacuolar protein sorting–associated protein

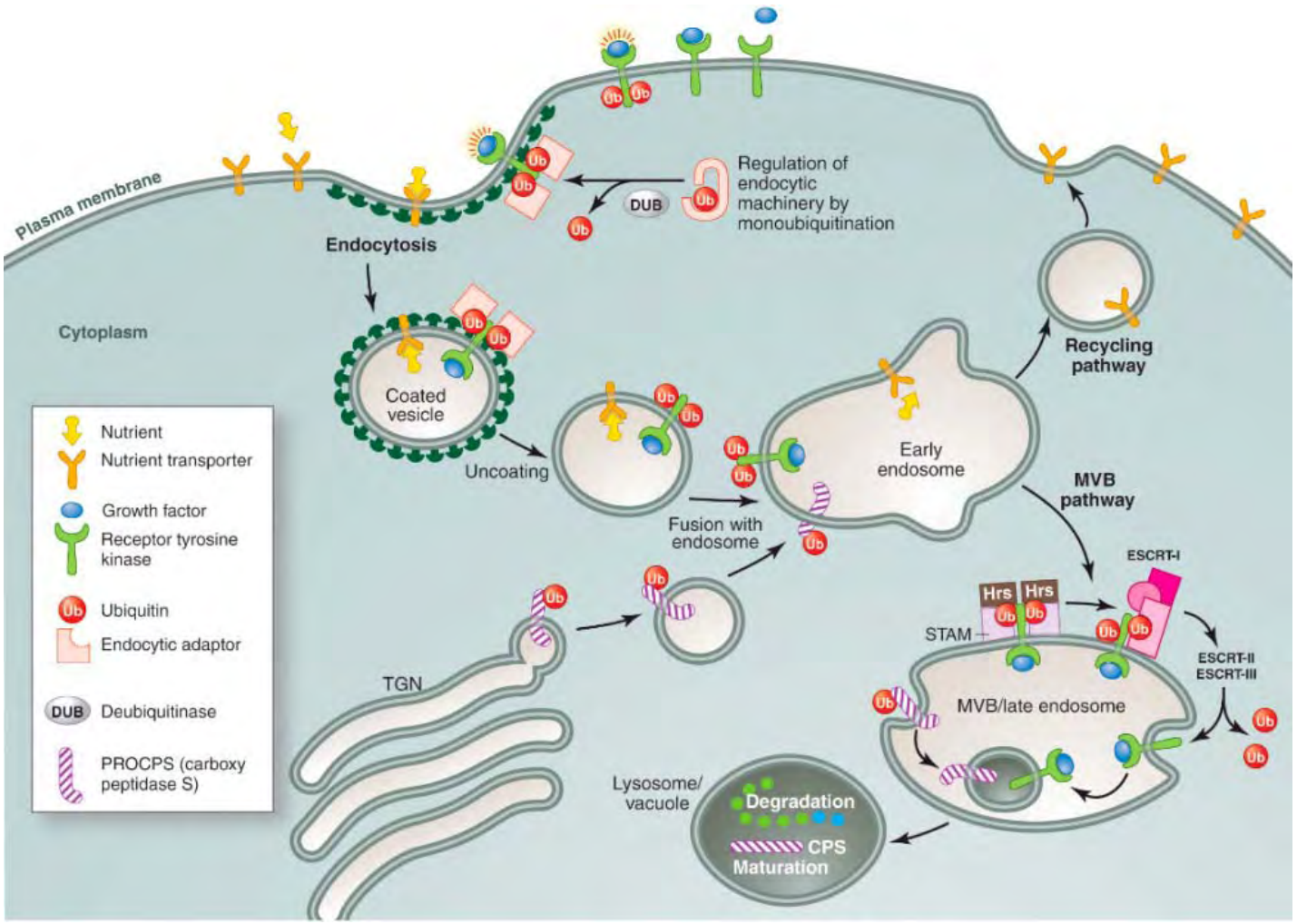

Fig. 1. Ub plays a major role at two steps of the secretory/endocytic pathways. Many plasma membrane proteins and receptors are ubiquitinated on their cytoplasmic domains. In animal cells, this may affect the choice of endocytic pathway (not shown). Ub also acts as a signal into the MVB pathway, where Ub is recognized by Vps27/Hrs and passed onto ESCRT complexes. Ub is removed before entry into internal vesicles of the MVB. Non-ubiquitinated proteins, such as nutrient transporters, recycle from endosomes back to the cell surface. TGN, trans-Golgi network; STAM, signal-transducing adaptor molecule.

(Vps27) complex through its Ub-interaction motif domains, and the complex is anchored on endosomes via the Vps27 FYVE domain, which binds phosphatidylinositol (PI) 3-phosphate (*29*). The Vps27 complex and ubiquitinated substrates are then thought to interact with ESCRT-I complex, from where the ubiquitinated cargoes are passed on to ESCRT-II and then to ESCRT-III, which is required for the concentration of cargoes into the MVB vesicles. ESCRT-III also regulates the accessory factors Bro1p and Doa4p, a Ub hydrolase that removes Ub from the cargo before it is sequestered into the MVB vesicles. Removal of Ub before receptor incorporation into MVBs is not mandatory because Ub that is fused in frame with cargo molecules gets sorted into MVBs, but such removal seems to be required for the maintenance of the free Ub pool, upon which receptor trafficking depends. Recent findings point to a regulatory role of deubiquitination in receptor down-regulation, because depletion of Ub hydrolases by RNA interference caused accelerated degradation of ligand-activated receptors (*30*, *31*). The topology of MVB vesicle budding is similar to biosynthetic viral budding from the cell surface, and several components are conserved (*32*).

Viruses also exploit the Ub-dependent entry into MVBs to escape immune recognition. Down-regulation of the major histocompatibility complex I (MHC I) by Kaposi's sarcoma–associated herpesvirus (KSHV) involves a virally encoded E3 Ub ligase that associates with MHC I molecules in the late secretory pathway, recruiting host Ub-conjugating enzymes to modify MHC class I molecules with Ub^{K63} chains. This ubiquitination targets the MHC I molecules for degradation through the MVB pathway (*33*).

Protein Regulation by Monoubiquitination

Many of the UBD-containing proteins that interact with ubiquitinated targets are themselves monoubiquitinated, especially the endocytic adaptor proteins (*8*). Insight into the role of monoubiquitination has recently been obtained (*34*). Sts1 and Sts2 bind through their N-terminal UBDs to ubiquitinated EGFR complexes and inhibit their endocytosis. Monoubiquitination of Sts2 leads to an intramolecular interaction between the UBD and its Ub, preventing Sts2 from interacting with exogenous Ub and EGFR. This intramolecular inhibitory reaction is functionally relevant because an Sts2-Ub chimera caused a substantial increase in EGFR down-regulation. Other endocytic adaptors with UBDs, such as Eps15 and hepatocyte growth factor–regulated tyrosine kinase substrate (Hrs), showed similar intramolecular interactions with their monoubiquitin. Only a small fraction of these proteins are monoubiquitinated at any one time (*34*, *35*), but this would be consistent with a regulatory role, where monoubiquitination of the UBD-containing proteins could act as a conformational switch, promoting dissociation from their targets. Once dissociated, the proteins could be rapidly deubiquitinated and reused. Therefore, changes in ubiquitination status could act as a rapid switch between active and inactive forms, permitting the highly dynamic exchange of ubiquitinated cargoes between the UBD-harboring proteins that is required by the endocytic sorting machinery.

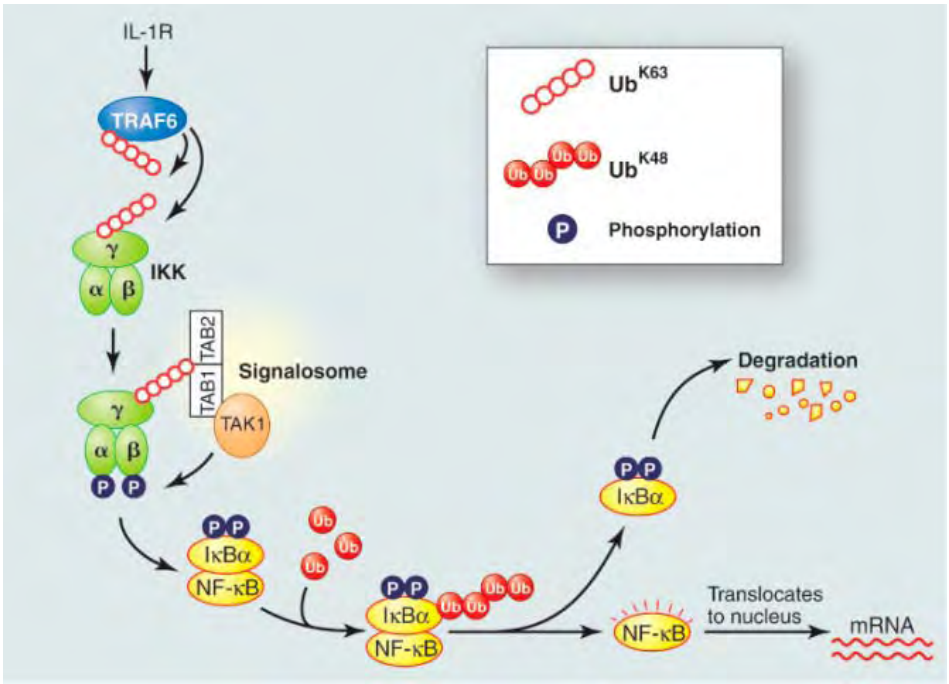

Fig. 2. Activated IL-1 receptor (IL-1R) stimulates TRAF6-dependent Ub^{K63} polyubiquitination of IKKγ (NEMO). The TAB/TAK1 complex is recruited, forming a "signalosome" that phosphorylates IKK, which in turn phosphorylates IκB, making it a substrate for Ub^{K48} polyubiquitination and subsequent degradation by the proteasome (Fig. 3). NF-κB is free to migrate to the nucleus and induce transcription.

Role of Ub in Signaling

Ubiquitination has been found to serve as a signal for directing internalization of signaling receptors and their incorporation into MVBs for targeting to lysosomal degradation. However, several receptors can transmit signals from endocytic compartments, and these signals might be qualitatively different from those initiated at the plasma membrane. For example, TrkA promotes NGF-mediated cell survival at the cell surface, whereas it induces differentiation when internalized (*36*). TrkA is modified by Ub^{K63} polyubiquitination upon stimulation with NGF in a p75 neurotrophin receptor–dependent manner. This modification is necessary for TrkA internalization and TrkA signaling from endosomes because abrogation of ubiquitination prevents NGF-mediated differentiation of neuronal cells (*26*). TrkA is multiubiquitinated by Nedd4-2, an E3 ligase, in an NGF-dependent manner, which is important for down-regulating the levels of activated receptor (*37*). Unexpectedly, this multiubiquitination did not show any effect on TrkA internalization. One can speculate a hierarchy of differential ubiquitination, whereby Ub^{K63} chains promote internalization while multiubiquitination regulates receptor degradation.

Monoubiquitination and endocytosis are prerequisites for activation and signaling of Notch receptor, which is a regulator of cell development and cell fate in metazoans (*38*). Recently, it has emerged that the ubiquitination of Notch ligands Delta and Serrate (type I membrane proteins and ligands of Notch) is necessary for their endocytosis in signal-sending cells and thus is a key event in activating the Notch cascade in the signal-receiving cells (*39*). Presently, the mechanism by which endocytosis of Notch ligands activates Notch signaling in neighboring cells remains elusive. An attractive model for the role of ubiquitination in regulating activity of the Notch ligands has been proposed. Ubiquitination is required for ligand activation but also makes the ligand prone to degradation. This would prevent the endless recycling of activated ligand and thereby limit the temporal extent of Notch-pathway activation (*39*). Thus, ubiquitination could couple activation with down-regulation, which allows for precise temporal control of signaling by limiting the lifetime of the activated signaling components.

Ub, Replication, and Transcription

Proliferating cell nuclear antigen (PCNA) is a replicative processivity factor that undergoes two types of Ub modifications. PCNA forms a polymerase sliding clamp around DNA, and the type of ubiquitination influences DNA-polymerase recruitment. Monoubiquitination of PCNA seems to shift recruitment from replica-

tive to translesion polymerases (*40*, *41*), which are error prone, whereas Ub^{K63} modification signals error-free DNA repair. The same residue in PCNA is also sumoylated, which is involved in normal DNA synthesis during S phase (*42*). Modification of PCNA with monoubiquitin or Ub^{K63} chains may thus regulate the engagement of low-fidelity lesion-bypass polymerases or high-fidelity replicases to the site of replication (*43*).

Ubiquitination has come to light as a major regulator of the nuclear factor (NF) κB signaling pathway. NF-κB transcription factors are activated by the proinflammatory cytokines [tumor necrosis factor–α (TNF-α) and interleukin-1β (IL-1β)], resulting in degradation of an inhibitor of NF-κB (IκB), which binds to NF-κB and retains it in the cytoplasm (Fig. 2). IκB is phosphorylated by a kinase [IκB kinase (IKK)], which has a regulatory subunit called NF-κB essential modulator (NEMO). NEMO is modified by Ub^{K63} polyubiquitination by the TNF-α receptor–associated factor 6 (TRAF6) (*44*). Ub^{K63} on NEMO activates IKK, which phosphorylates IκB, targeting it for polyubiquitination and proteasomal degradation (*45*). This action liberates NF-κB. Another kinase that activates IKK is TGF-β–activated kinase (TAK1). TAK1-binding proteins (TAB1, TAB2, and TAB3) bind to TRAF6 and TAK1, stimulating its kinase activity, and preferentially bind Ub^{K63} (*46*). Furthermore, the TRAF6-TAB2-TAK1 complex interacts with IKK. So, Ub^{K63} may function as part of a scaffold to assemble a TAK1-IKK–containing signaling complex (*47*). When NF-κB activation is induced by TNF-α, receptor-interacting protein (RIP), which is important for receptor complex assembly, undergoes Ub^{K63} polyubiquitination. NEMO binds to Ub^{K63}-polyubiquitinated RIP and thereby recruits IKK to the TNF receptor. This recruitment could explain the propagation of signal from the activated TNF receptor to the activation of IKK and NF-κB. NEMO also stabilizes RIP through interaction with its Ub^{K63} chains, and thus the binding of NEMO could impair RIP interaction with another protein, A20 (*48*). A20 inhibits NF-κB signaling by sequentially removing Ub^{K63} chains from RIP and targeting it for degradation through ligation of Ub^{K48} chains (*6*).

In general, it is assumed that Ub^{K48} polyubiquitin chains act to target modified substrates for proteasomal degradation and that Ub^{K63} linkages are used for other functions. However, for a budding yeast transcription factor Met4p, Ub^{K48} polyubiquitination represses its activity but does not affect Met4p stability (Fig. 3) (*49*). Further work on Met4p has shown that it contains a UBD, which interacts in cis with the Ub^{K48} chains, preventing their elongation. Because multiple Ub^{K48} linkages are required for recognition by the proteasome (*50*), this leads to protection from proteolysis. In this case, Ub^{K48} chains act to switch Met4p to an inactive state but do not lead to Met4p degradation. Rapid deubiquitination of Met4p, in response to stimulus, recreates a large pool of active Met4p and thus allows a rapid transcriptional response to changing environmental conditions (*51*).

Ubiquitination and Disease

Ubiquitination is a widely used posttranslational modification to regulate cellular processes. Thus, aberrations in the ubiquitination system lead to disease and malignancies. Enhanced degradation of tumor suppressor proteins by the proteasomal pathway or abrogation of degradation of oncogenic proteins is the basis of oncogenesis in several cancers. Several pathogens and viruses have evolved mechanisms to subvert the Ub system for their own ends. For instance, anthrax toxin triggers ubiquitination of its receptor to facilitate efficient and rapid endocytosis of the toxin-receptor complex. Endocytosis of the toxin-receptor complex, in turn, is important for toxin action, because passage through low-pH endosomal compartments makes the toxin competent to induce toxicity in cells (*52*). *Yersinia* produces a virulence factor, YopJ, that acts as a deubiquitination enzyme, preventing activation of NF-κB (*53*). On the other hand, many viruses encode for E3 Ub ligases in order to down-regulate proteins of the immune pathway. The E3 Ub ligase, named modulator of immune recognition 1 of KSHV, can mediate ubiquitination of cysteine residues in the MHC class I molecule. This form of ubiquitination is found to be sufficient for endocytosis and degradation of MHC I molecules (*54*). The finding that nonlysine residues are also targets of ubiquitination extends the number of potential substrates to molecules that do not contain an accessible Lys or an accessible N terminus. Also, transient ubiquitination of substrates may occur and may be harder to detect than other forms of ubiquitination because thiol ester bonds of Ub-cysteine are more labile than isopeptide bonds of Ub-Lys. Thus, the regulatory processes rendered by Ub might be more complex than previously thought.

Liddle's syndrome, which affects sodium transport, is a disease in which ubiquitination clearly plays a role in regulating sodium channel activity by controlling its down-regulation via endocytosis (*55*). Ub is also thought to play a role in neurodegenerative diseases. Accumulation of insoluble proteinaceous deposits enriched with Ub and components

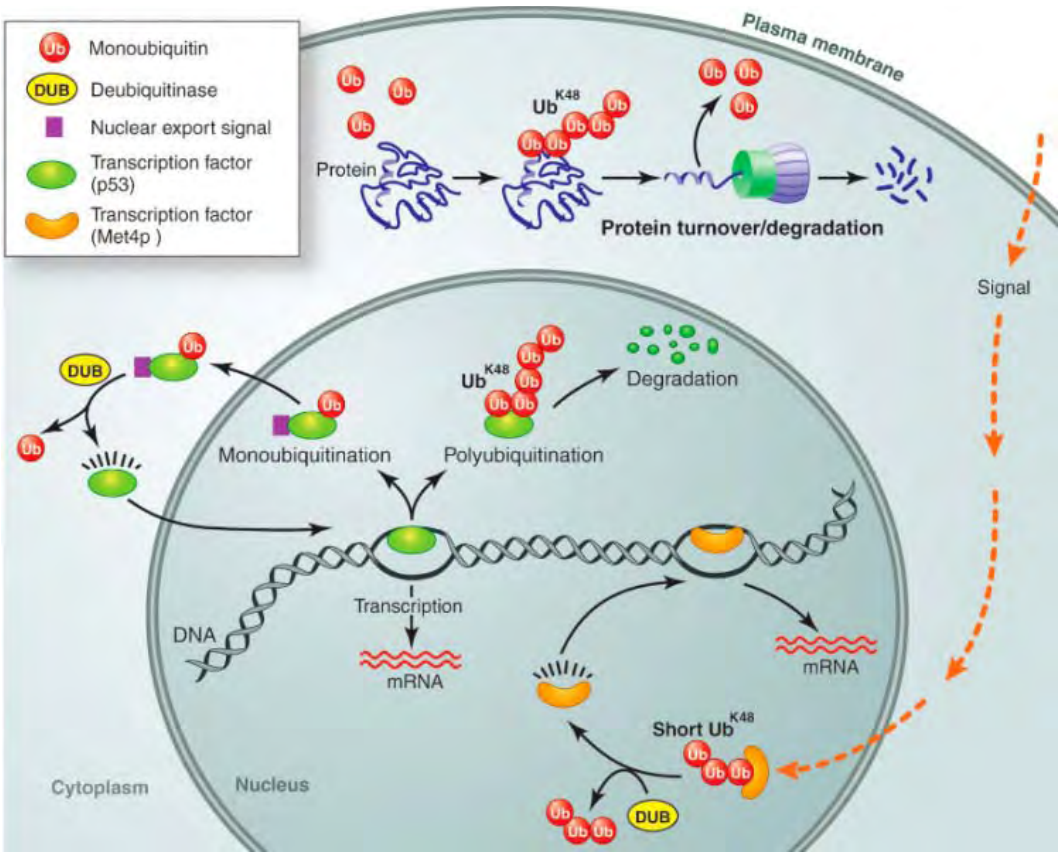

Fig. 3. Protein modified by long (more than four ubiquitins) Ub^{K48} chains is targeted to the proteasome for degradation. Short Ub^{K48} chains can inhibit activity of a transcription factor (Met4p) without leading to its degradation. Deubiquitination activates the transcription factor. A transcription factor (p53) is imported into the nucleus. Monoubiquitination leads to its nuclear export, whereas polyubiquitination leads to its degradation (*59*).

of the Ub-proteasome system has been reported in a broad array of human neurodegenerative disorders. Notably in Parkinson's disease, defects in ubiquitination are thought to play a crucial role in generation of the disease state. Parkin, a protein implicated in familial Parkinson's disease, is an E3 Ub ligase that contains a Ub-like domain that interacts in a regulated manner with the UBD of Eps15. This interaction seems to negatively affect EGFR endocytosis and down-regulation and to promote signaling through PI 3-kinase–Akt, because parkin mutant cells show increased EGFR down-regulation (*56*), and this could have an effect on progression of the disease. Parkin has also been found to synthesize Ub^{K63} polyubiquitin chains on proteins, including synphilin-1, and on the modified proteins that are found in Lewy-like inclusion bodies (*57*). Considering the suggested neuroprotective role of inclusion bodies, it is tempting to speculate that Ub^{K63} modification represents a protective route by which unfolded or aggregated proteins could be diverted away from an overloaded proteasomal machinery.

Ubiquitination may also provide an entry point for therapy against previously difficult targets. Myc is a critical transcription factor that is misregulated in tumors but is a difficult chemotherapeutic target because it has no enzymatic activity. Ub^{K63} is added to Myc by the E3 ligase HectH9, and the modification is essential for tumor proliferation (*58*). As an enzyme, HectH9 should be a more suitable target than Myc for chemotherapy.

Conclusions

Ub is a chemically complex molecule that offers a large surface area to interact with other proteins and can form chains with distinct conformations, which makes it a very versatile modification. These properties of Ub make it an ideal regulator of very diverse biological processes. The multiple steps required for protein ubiquitination, the specificity involved, and the process of deubiquitination make it subject to control at many levels. We are only at the beginning of understanding the wide array of Ub functions in cellular processes. Protein ubiquitination is best compared to protein phosphorylation. In both cases, proteins are specifically, diversely, and reversibly modified. The modifications can directly affect activity or conformation but can also act as interaction surfaces to recruit other proteins containing domains that recognize the specific modification. The assemblies then confer function to the modification. Understanding the functions of proteins that recognize ubiquitinated substrates will be the key to understanding the function of Ub in a particular cellular process. Also, because of the similarities between ubiquitination and phosphorylation, it is likely that ubiquitination can positively or negatively affect cellular processes, depending on the particular substrate and the context. Care should thus be taken when comparing experiments performed in different contexts. Finally, the fact that many proteins that are targets for ubiquitination are also phosphorylated increases the complexity of regulation that can be achieved.

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Haploid Females in the Parasitic Wasp *Nasonia vitripennis*

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Haplodiploidy, a sex determination mechanism, occurs in rotifers, nematodes, mites, and insects. The order Hymenoptera consists almost entirely of haplodiploid species, in which haploid males develop from unfertilized eggs and diploid females develop from fertilized eggs. In complementary sex determination (CSD), sex is determined by the allelic state of one locus with multiple alleles (*1*). Females are heterozygous, and males receive a single chromosome set from their mother. Diploid males can result under CSD by inbreeding, but they are mostly infertile (*2*). The parasitic wasp *Nasonia vitripennis* Walker does not have CSD and inbreeds readily. No cases of haploid females among Hymenoptera have been found previously. We report that haploid females can develop in *N. vitripennis*.

We found a Canadian strain in which ~10% of unfertilized eggs developed into gynandromorphs, that is, individuals with both male and female features. Their frequency was increased by both directional selection (~20%) and high culturing temperature (~40%). Females differ externally from males in the dark color of antennae and legs, large wings, and presence of an ovipositor. Gynandromorphs show an anterior-posterior gradient of feminization ranging from individuals with only female antennae to individuals with complete female morphology (parthenogenetic females). Tetracycline used to remove *Wolbachia* (and other bacterial symbionts) did not affect gynandromorph frequency.

Ploidy level of gynandromorphs and parthenogenetic females was determined in 459 eggs at 4, 8, 12, and 26 hours of development and in brain tissue of 99 4-day-old larvae. No diploids were found, although 40% were expected on the basis of emerging control wasps. Rare diploid cells (<1%) were observed at frequencies similar to those of normal haploid males. Flow cytometric analysis showed that all gynandromorphic individuals and parthenogenetic females contained the haploid amount of DNA (Fig. 1). The diploid *Nasonia* genome was estimated at 312 Mb, consistent with previous reports (*3*) and confirming that parthenogenetic females are haploid and do not arise by chromosome duplication in unfertilized eggs.

Parthenogenetic females have low fertility; only 2 of 281 females produced offspring (one son and two diapause larvae, respectively). Normal diploid females ($n = 24$) have four ovarioles on either abdominal side. Ovariole number in parthenogenetic females ($n = 17$) varies, averaging 3.6 (range from 1 to 5) on either side. Diploid females carried on average 28.8 ± 1.8 SE eggs upon emergence (Fig. 1B), whereas haploid females had only 4.2 ± 0.7 SE eggs (Fig. 1D). Oogenesis proceeds normally in haploid females. However, instead of arresting in first prophase, as in diploid females, mature eggs in haploid females consistently showed five condensed chromosomes that appeared to have proceeded further into the meiotic cycle (Fig. 1, B and D), which may account for the sterility of haploid females. Gynandromorphs with a male abdomen had normal testes (Fig. 1, A and C) and live sperm.

Previously only a mite species had been reported to produce haploid females among the higher metazoans (*4*). Parthenogenetic *Nasonia* females have severely reduced fecundity and fertility. In contrast, diploid *Nasonia* males are fully fertile and produce diploid sperm mitotically, indicating that ploidy is more important for female than male germline development. These findings argue against female development depending on paternally inherited chromosomes, but are consistent (along with other data) with a maternal imprinting model for *Nasonia* sex determination (*5*).

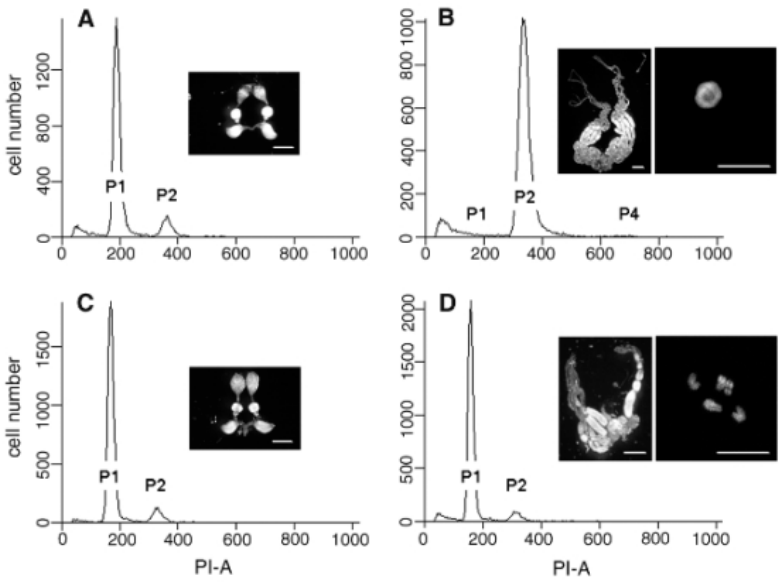

Fig. 1. Flow cytometry and photographs of ovaries and testes of *Nasonia*. (**A**) Normal haploid male. (**B**) Normal diploid female. (**C**) Gynandromorph with female antennae and wings but male abdomen. (**D**) Parthenogenetic female. Peak P1 corresponds to haploid, P2 to diploid, and P4 to tetraploid cells. Some endopolyploidization is evident in males (P2) and females (P4). PI-A indicates propidium iodide area as a measure for DNA content. Photo inserts in (A) and (C) are testes; for (B) and (D), ovaries and meiosis in eggs. Scale bars indicate 150 μm in testes and ovaries and 40 μm in eggs.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5809/206/DC1
Materials and Methods
Fig. S1

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Draft Genome Sequence of the Sexually Transmitted Pathogen *Trichomonas vaginalis*

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We describe the genome sequence of the protist *Trichomonas vaginalis*, a sexually transmitted human pathogen. Repeats and transposable elements comprise about two-thirds of the ~160-megabase genome, reflecting a recent massive expansion of genetic material. This expansion, in conjunction with the shaping of metabolic pathways that likely transpired through lateral gene transfer from bacteria, and amplification of specific gene families implicated in pathogenesis and phagocytosis of host proteins may exemplify adaptations of the parasite during its transition to a urogenital environment. The genome sequence predicts previously unknown functions for the hydrogenosome, which support a common evolutionary origin of this unusual organelle with mitochondria.

Trichomonas vaginalis is a flagellated protist that causes trichomoniasis, a common but overlooked sexually transmitted human infection, with ~170 million cases occurring annually worldwide (*1*). The extracellular parasite resides in the urogenital tract of both sexes and can cause vaginitis in women and urethritis and prostatitis in men. Acute infections are associated with pelvic inflammatory disease, increased risk of human immunodeficiency virus 1 (HIV-1) infection, and adverse pregnancy outcomes. *T. vaginalis* is a member of the parabasilid lineage of microaerophilic eukaryotes that lack mitochondria and peroxisomes but contain unusual organelles called hydrogenosomes. Although previously considered to be one of the earliest branching eukaryotic lineages, recent analyses leave the evolutionary relationship of parabasalids to other major eukaryotic groups unresolved (*2*, *3*). In this article, we report the draft sequence of *T. vaginalis*, the first parabasalid genome to be described.

Genome structure, RNA processing, and lateral gene transfer. The *T. vaginalis* genome sequence, generated using whole-genome shotgun methodology, contains 1.4 million shotgun reads assembled into 17,290 scaffolds at ~7.2× coverage (*4*). At least 65% of the *T. vaginalis* genome is repetitive (table S1). Despite several procedures developed to improve the assembly (*4*), the superabundance of repeats resulted in a highly fragmented sequence, preventing investigation of *T. vaginalis* genome architecture. The repeat sequences also hampered measurement of genome size, but we estimate it to be ~160 Mb (*4*). A core set of ~60,000 protein-coding genes was identified (Table 1), endowing *T. vaginalis* with one of the highest coding capacities among eukaryotes (table S2). Introns were identified in 65 genes, including the ~20 previously documented (*5*). Transfer RNAs (tRNAs) for all 20 amino acids were found, and ~250 ribosomal DNA (rDNA) units were identified on small contigs and localized to one of the six *T. vaginalis* chromosomes (Fig. 1).

The Inr promoter element was found in ~75% of 5′ untranslated region (UTR) sequences (*4*), supporting its central role in gene expression (*6*). Intriguingly, the eukaryotic transcription machinery of *T. vaginalis* appears more metazoan than protistan (table S3 to S5). The presence of a *T. vaginalis* Dicer-like gene, two Argonaute genes, and 41 transcriptionally active DEAD-DEAH-box helicase genes suggests the existence of an RNA interference (RNAi) pathway (fig. S1). Identification of these components raises the possibility of using RNAi technology to manipulate *T. vaginalis* gene expression.

During genome annotation, we identified 152 cases of possible prokaryote-to-eukaryote lateral gene transfer (LGT) [tables S6 and S7 and Supporting Online Material (SOM) text], augmenting previous reports of conflicting phylogenetic relationships among several enzymes (*7*). The putative functions of these genes are diverse, affecting various metabolic pathways (fig. S2) and strongly influencing the evolution of the *T. vaginalis* metabolome. A majority (65%) of the 152 LGT genes encode metabolic enzymes, more than a third of which are involved in carbohydrate or amino acid metabolism (Fig. 2). Several LGT genes may have been acquired from Bacteroidetes-related bacteria, which are abundant among vertebrate intestinal flora (fig. S3).

Repeats, transposable elements, and genome expansion. The most common 59 repeat families identified in the assembly (*4*) constitute ~39 Mb of the genome and can be classified as (i) virus-like; (ii) transposon-like, including ~1000 copies of the first *mariner* element identified outside animals (*8*); (iii) retrotransposon-like; and (iv) unclassified (Table 2). Most of the

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59 repeats are present in hundreds of copies (average copy number ~660) located on small (1- to 5-kb) contigs, and each repeat family is extraordinarily homogenous, with an average polymorphism of ~2.5%.

The lack of a strong correlation between copy number and average pairwise difference between copies (fig. S4) suggests that a sudden expansion of the repeat families had occurred. To estimate the time of expansion, we compared the degree of polymorphism among *T. vaginalis* repeats to the divergence between *T. vaginalis* and its sister taxon *T. tenax*, a trichomonad of the oral cavity (*9*), for several protein-coding loci (*4*). Our results indicate that repeat family amplification occurred after the two species split (table S8). Several families have also undergone multiple expansions, as implied by bi- or trimodal distribution of pairwise distances between copies (fig. S5). *T. vaginalis* repeat families appear to be absent in *T. tenax* but are present in geographically diverse *T. vaginalis* (*4*), consistent with the expansion having occurred after speciation but before diversification of *T. vaginalis*.

Table 1. Summary of the *T. vaginalis* genome sequence data. Assembly size (bp, base pairs) includes all contigs and differs from estimated genome size of ~160 Mb (*4*). The scaffold size is the minimum scaffold length, such that more than half the genome is contained in scaffolds of at least that length. The number of predicted genes may include low-complexity repeats or novel transposable elements rather than true *T. vaginalis* genes, but in the absence of decisive evidence these remain in the gene set. The number of evidence-supported genes includes those with either similarity to a known protein ($E < 1 \times 10^{-10}$, >25% length of protein) or similarity to an expressed sequence tag (>95% identity over >90% length of the gene). A total of 763 rDNA fragments (258 copies of 28S, 254 copies of 18S, and 251 copies of 5.8S) were identified.

Feature	Value
Genome	
Size of assembly (bp)	176,441,227
G+C content (%)	32.7
No. of scaffolds	17,290
N_{50} scaffold size (bp)	68,338
Protein-coding genes	
No. of predicted genes	59,681
No. of evidence-supported genes	25,949
No. of genes with introns	65
Mean gene length (bp)	928.6
Gene G+C content (%)	35.5
Gene density (bp)	2956
Mean length of intergenic regions (bp)	1165.4
Intergenic G+C content (%)	28.8
Non–protein-coding genes	
Predicted tRNA genes	479
Predicted 5.8S, 18S, and 28S rDNA units	~250

The large genome size, high repeat copy number, low repeat polymorphism, and evidence of repeat expansion after *T. vaginalis* and *T. tenax* diverged suggest that *T. vaginalis* has undergone a very recent and substantial increase in genome size. To determine whether the genome underwent any large-scale duplication event(s), we analyzed age distributions of gene families with five or fewer members (*4*). A peak in the age distribution histogram of pairs of gene families was observed (fig. S6), indicating that the genome underwent a period of increased duplication, and possibly one or more large-scale genome duplication events.

Metabolism, oxidative stress, and transport. *T. vaginalis* uses carbohydrate as a main energy source via fermentative metabolism under aerobic and anaerobic conditions. We found the parasite to use a variety of amino acids as energy substrates (Fig. 2) (*10*), with arginine dihydrolase metabolism a major pathway for energy production (fig. S7) (*11*). We confirmed a central role for aminotransferases (Fig. 2 and table S9) and glutamate dehydrogenase as indicated previously (*12*, *13*); these pathways are likely catabolic but may be reversible to allow the parasite to synthesize glutamate, aspartate, alanine, glutamine, and glycine. Genes required for synthesis of proline from arginine (fig. S7) and for threonine metabolism (fig. S8) were identified. We also identified a de novo biosynthesis pathway for cysteine via cysteine synthase, an LGT candidate (fig. S8) (*14*), and genes encoding enzymes involved in methionine metabolism, including its possible regeneration (fig. S9).

Earlier studies indicated that de novo lipid biosynthesis in *T. vaginalis* is confined to the major phospholipid phosphatidylethanolamine (PE) (*15*), whereas other lipids, including cholesterol, are likely acquired from exogenous sources. We found an absence of several essential enzyme-encoding genes in the synthesis and degradation pathways of nearly all lipids (*4*), in contrast to the PE synthetic pathway, which appears complete; however, experimental verification of these results is required.

T. vaginalis is microaerophilic with a primarily anaerobic life style and thus requires redox and antioxidant systems to counter the detrimental effects of oxygen. Genes encoding a range of defense molecules, such as superoxide dismutases, thioredoxin reductases, peroxiredoxins, and rubrerythrins, were identified (table S10).

T. vaginalis demonstrates a broad range of transport capabilities, facilitated by expansion of particular transporter families, such as those for sugar and amino acids (table S11). The parasite also possesses more members of the cation-chloride cotransporter (CCC) family than any other sequenced eukaryote, likely reflecting osmotic changes faced by the parasite in a mucosal environment.

None of the proteins required for glycosylphosphatidylinositol (GPI)-anchor synthesis were identified in the genome sequence, making *T. vaginalis* the first eukaryote known to lack an apparent GPI-anchor biosynthetic pathway. Whether *T. vaginalis* has evolved an unusual biosynthetic pathway for synthesis of its nonprotein lipid anchors, such as the inositolphosphoceramide of surface lipophosphoglycans (*16*), remains to be determined.

Massively expanded gene families. Many gene families in the *T. vaginalis* genome have undergone expansion on a scale unprecedented in unicellular eukaryotes (Table 3). Such "conservative" gene family expansions are likely to improve an organism's adaptation to its environment (*17*). Notably, the selective expansion of subsets of the membrane trafficking machinery, critical for secretion of pathogenic proteins, endocytosis of host proteins, and phagocytosis of bacteria and host cells (table S12), correlates well with the parasite's active endocytic and phagocytic life-style.

Massively amplified gene families also occur in the parasite's kinome, which comprises ~880 genes (SOM text) encoding distinct eukaryotic protein kinases (ePKs) and ~40 atypical protein

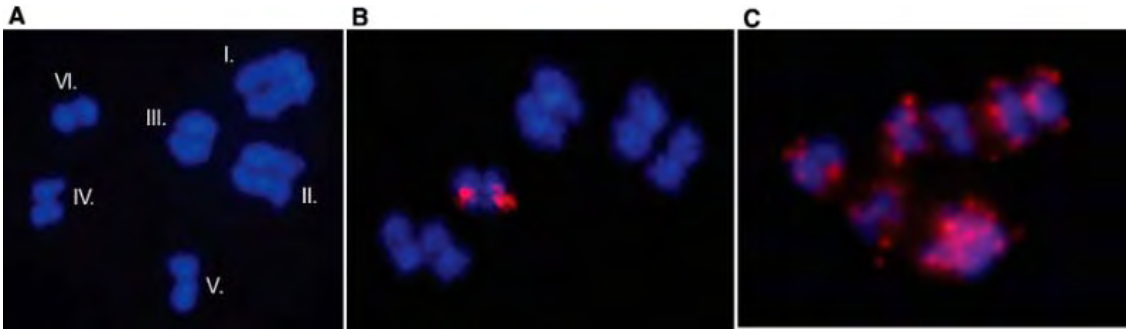

Fig. 1. Karyotype and fluorescent in situ hybridization (FISH) analysis of *T. vaginalis* chromosomes. (**A**) Metaphase chromosome squashes of *T. vaginalis* reveal six chromosomes (I to VI). (**B**) FISH analysis using an 18S rDNA probe shows that all ~250 rDNA units localize to a single chromosome. (**C**) In contrast, the *Tvmar1* transposable element (*8*) is dispersed throughout the genome.

kinases, making it one of the largest eukaryotic kinomes known. The parasite has heterotrimeric guanine nucleotide–binding proteins and components of the mitogen-activated protein kinase (MAPK) pathway, suggesting yeast-like signal transduction mechanisms. Unusually, the *T. vaginalis* kinome contains 124 cytosolic tyrosine kinase–like (TKL) genes, yet completely lacks receptor serine/threonine ePKs of the TKL family. Inactive kinases were found to make up 17% of the *T. vaginalis* kinome (table S13); these may act as substrates and scaffolds for assembly of signaling complexes (*18*). ePK accessory domains are important for regulating signaling pathways, but just nine accessory domain types were identified in 8% (72/883) of the *T. vaginalis* ePKs (table S14), whereas ~50% of human ePKs contain at least 1 of 83 accessory domain types. This suggests that regulation of protein kinase function and cell signaling in *T. vaginalis* is less complex than that in higher eukaryotes, a possible explanation for the abundance of *T. vaginalis* ePKs.

T. vaginalis possesses several unusual cytoskeletal structures: the axostyle, the pelta, and the costa (*19*). Most actin- and tubulin-related components of the cytoskeleton are present (table S15), with the exception of homologs of the actin motor myosin. In contrast, homologs of the microtubular motors kinesin and dynein are unusually abundant (Table 3). Thus, *T. vaginalis* intracellular transport mechanisms are mediated primarily by kinesin and cytoplasmic dynein, as described for *Dictyostelium* and filamentous fungi, raising the possibility that the loss of myosin-driven cytoplasmic transport is not uncommon in unicellular eukaryotes (*20*). Whether the structural remodeling of amoeboid *T. vaginalis* during host colonization (see below) is actin-based, as described for other eukaryotes, or driven by novel cytoskeletal rearrangements remains an open question.

We identified homologs of proteins involved in DNA damage response and repair, chromatin restructuring, and meiosis, the latter a process not thought to occur in the parasite (table S16). Of the 29 core meiotic genes found, several are general repair proteins required for meiotic progression in other organisms (*21*), and eight are meiosis-specific proteins. Thus, *T. vaginalis* contains either recent evolutionary relics of meiotic machinery or genes functional in meiotic recombination in an as-yet undescribed sexual cycle.

Molecular mechanisms of pathogenesis. *T. vaginalis* must adhere to host cells to establish and maintain an infection. A dense glycocalyx composed of lipophosphoglycan (LPG) (Fig. 3) and surface proteins has been implicated in adherence (*22*), but little is known about this critical pathogenic process. We identified genes encoding enzymes predicted to be required for LPG synthesis (table S17). Of particular interest are the genes required for synthesis of an unusual nucleotide sugar found in *T. vaginalis* LPG, the monosaccharide rhamnose, which is absent in the human host, making it a potential drug target. Genes (some of which are LGT candidates) were identified that are involved in sialic acid biosynthesis, consistent with the reported presence of this sugar on the parasite surface (*23*).

We identified eight families containing ~800 proteins (*4*) that represent candidate surface molecules (Fig. 3 and table S18), including ~650 highly diverse BspA-like proteins characterized by the *Treponema pallidum* leucine-rich repeat, TpLLR. BspA-like proteins are expressed on the surface of certain pathogenic bacteria and mediate cell adherence and aggregation (*24*). The only other eukaryote known to encode BspA-like proteins, the mucosal pathogen *Entamoeba histolytica* (*25*), contains 91 such proteins, one of which was recently localized to the parasite surface (*26*).

There are >75 *T. vaginalis* GP63-like proteins, homologs of the most abundant surface proteins of *Leishmania major*, the leishmanolysins, which contribute to virulence and pathogenicity through diverse functions in both the insect vector and the mammalian host (*27*). Most *T. vaginalis* GP63-like genes possess the domains predicted to be required for a catalytically active metallopeptidase, including a short HEXXH motif (*28*) (Fig. 3). Unlike trypanosomatid GP63 proteins, which are predicted to be GPI-anchored, most *T. vaginalis* GP63-like proteins have a predicted C-terminal transmembrane domain as a putative cell surface anchor, consistent with the apparent absence of GPI-anchor biosynthetic enzymes. Other *T. vaginalis* protein families share domains with *Chlamydia* polymorphic membrane proteins, *Giardia lamblia* variant surface proteins, and *E. histolytica* immunodominant variable surface antigens (Fig. 3 and table S18).

After cytoadherence, the parasite becomes amoeboid, increasing cell-to-cell surface contacts and forming cytoplasmic projections that interdigitate with target cells (*19*). We have identified genes encoding cytolytic effectors, which may be released upon host-parasite contact. *T. vaginalis* lyses host red blood cells, presumably as a means of acquiring lipids and iron and possibly explaining the exacerbation of symptoms observed during menstruation (*29*). This hemolysis is dependent on contact, temperature, pH, and Ca^{2+}, suggesting the involvement of pore-forming proteins (*30*) that insert into the lipid bilayer of target cells, mediating osmotic lysis. Consistent with this, we have identified 12 genes (*TvSaplip1* to *TvSaplip12*) containing saposin-like (SAPLIP) pore-forming domains (fig. S10). These domains show a predicted six-cysteine pattern and abundant hydrophobic residues in conserved positions while displaying high sequence variability (fig. S11). The TvSaplips are similar to amoebapore proteins secreted by *E. histolytica* and are candidate trichopores that mediate a cytolytic effect.

The degradome. Peptidases perform many critical biological processes and are potential virulence factors, vaccine candidates, and drug targets (*31*). *T. vaginalis* contains an expanded degradome of more than 400 peptidases (SOM text), making it one of the most complex degradomes described (table S19). Of the three families of aspartic peptidases (table S20), *T. vaginalis* contains a single member of the HIV-1 retropepsin family that might serve as a putative candidate for anti-HIV peptidase inhibitors. Many studies have implicated papain

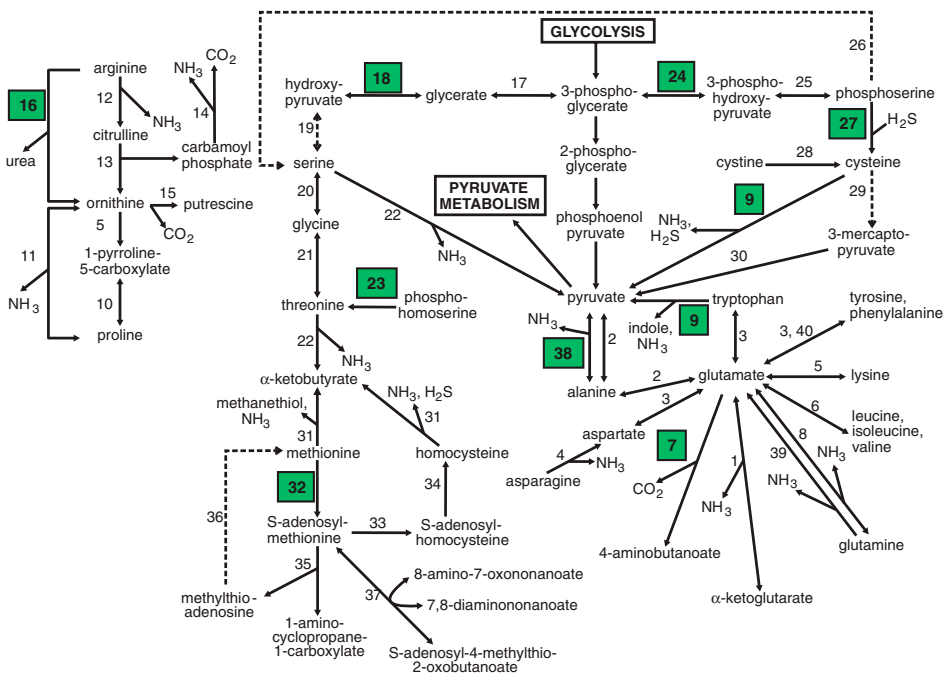

Fig. 2. Schematic of *T. vaginalis* amino acid metabolism. A complete description of enzymatic reactions (represented as numbers) is given in the SOM text. Broken lines represent enzymes for which no gene was identified in the genome sequence, although the activity would appear to be required. Green boxes indicate enzymes encoded by candidate LGT genes.

Table 2. Summary of highly repetitive sequences in the genome of *T. vaginalis*. The 31 unclassified repeat families have been collapsed into one group.

Repeat name	Putative identity	Copy no.	Length (bp)	Cumulative length (bp)	Average pairwise difference (%)
		Viral			
R128b	Poxvirus D5 protein	203	2348	370,658	1.2
R169a	Phage tail fiber prot	903	752	607,929	3.9
R1794	Hypothetical	2243	1037	1,879,762	3.0
R299a	KilA-N terminal domain	867	873	655,984	6.6
R3a	Poxvirus D5 protein	954	2637	1,721,187	4.6
R9a	KilA-N terminal domain	831	663	503,959	3.6
R947a	KilA-N terminal domain	518	669	298,778	3.1
Average (total)		931	1283	(6,038,257)	3.7
		Transposon			
R8	Mariner transposase	982	1304	1,158,473	0.5
R107	Integrase	384	2246	518,936	0.9
R119	Mutator-like profile	282	2954	303,256	1.1
R11b	Integrase	1842	981	1,634,764	2.1
R128a	HNH endonuclease	200	800	131,491	1.5
R130a	Mutator-like profile	173	1129	137,526	0.7
R165	Mutator-like profile	365	2410	368,152	1.1
R178	Integrase	580	2433	636,901	1.5
R204	Integrase	51	1323	51,185	1.6
R210	Mutator-like profile	75	2127	118,581	0.9
R2375	Endonuclease	566	765	329,717	5.4
R242b	Integrase	49	1151	45,564	2.5
R26b	Integrase	1148	1141	1,175,921	2.6
R289a	Integrase	54	1250	53,674	2.2
R309b	Integrase	56	1601	73,737	1.4
R414a	Integrase	37	909	27,708	1.4
R41b	Integrase	927	1184	807,711	2.5
R473	Integrase	19	1124	15,254	1.3
Integ.a95313	Integrase	68	1301	22,023	2.3
Average (total)		414	1481	(7,610,574)	1.8
		Retrotransposon			
R1407.RT	Reverse transcriptase	6	2349	6,833	1.0
copia.a38393	Copia-like profile	7	11001	16,504	7.5
Average (total)		6.5	6675	(23,337)	4.3
		31 unclassified families			
Average (total)		793	1131	(24,617,704)	2.6
Total all				38,289,872	
Average all		660	1450		2.5

Table 3. Large gene families in *T. vaginalis*. Only gene families, or aggregates of gene families mediating a given process, for which there are more than 30 members and that have been assigned a putative function are listed. (See SOM text for subfamily organization.) The small guanosine triphosphatases (GTPases) are the sum of Rab and ARF small GTPases only; all other GTPases are shown in table S12. ABC, ATP-binding cassette; MFS, major facilitator superfamily; MOP, multidrug/oligosaccharidyl-lipid/polysaccharide; AAAP, amino acid/auxin permease.

Gene family (functional unit)	Members
Protein kinases	927
BspA-like gene family	658
Membrane trafficking: small GTPases	328
rDNA gene cluster	~250
Cysteine peptidase (clan CA, family C19)	117
Membrane trafficking: vesicle formation	113
ABC transporter superfamily	88
GP63-like (leishmanolysin)	77
MFS transporter family	57
MOP flippase transporter family	47
Cysteine peptidase (clan CA, family C1)	48
AAAP transporter family	40
Dynein heavy chain	35
P-ATPase transporter family	33
Serine peptidase (clan SB, family S8)	33
Membrane trafficking: vesicle fusion	31

family cysteine peptidases as virulence factors in trichomonads; we identified >40 of them, highlighting the diversity of this family. Cysteine peptidases that contribute to the 20S proteosome (ubiquitin C-terminal hydrolases) are abundant (117 members, ~25% of the degradome), emphasizing the importance of cytosolic protein degradation in the parasite. *T. vaginalis* has nine NlpC/P60-like members (table S20; several of which are LGT candidates), which play a role in bacterial cell wall degradation and the destruction of healthy vaginal microflora, making the vaginal mucosa more sensitive to other infections.

We also identified many subtilisin-like and several rhomboid-like serine peptidases, candidates for processing *T. vaginalis* surface proteins. In addition to the first asparaginase-type of threonine peptidase found in a protist, 13 families of metallopeptidases were also identified, as well as three cystatin-like proteins, natural peptidase inhibitors, which may regulate the activity of the abundant papain-like cysteine peptidases (table S20).

The hydrogenosome. Several microaerophilic protists and fungi, including trichomonads and ciliates, lack typical mitochondria and possess double-membrane hydrogenosomes, which produce adenosine triphophate (ATP) and molecular hydrogen through fermentation of metabolic intermediates produced in the cytosol. Although the origin of these organelles has been controversial, most evidence now supports a common origin with mitochondria (*3*). Few genes encoding homologs of mitochondrial transporters, translocons, and soluble proteins were identified in the *T. vaginalis* genome (fig. S12), suggesting that its hydrogenosome has undergone reductive evolution comparable to other protists whose mitochondrial proteomes are reduced (e.g., *Plasmodium*). Because nuclear-encoded hydrogenosomal matrix proteins are targeted to the organelle by N-terminal presequences that are proteolytically cleaved upon import (*32*) similar to mitochondrial precursor proteins, we screened the genome for consensus 5- to 20-residue presequences containing ML(S/T/A)$X_{(1...15)}$R (N/F/E/XF), MSL$X_{(1...15)}$R(N/F/XF), or MLR(S/N)F (*28*) motifs. A total of 138 genes containing putative presequences were identified, 67% of which are similar to known proteins, primarily ones involved in energy metabolism and electron-transport pathways (fig. S13 and table S21).

The production of molecular hydrogen, the hallmark of the hydrogenosome, is catalyzed by an unusually diverse group of iron-only [Fe]-hydrogenases that possess, in addition to a conserved H cluster, four different sets of functional domains (fig. S14), indicating that hydrogen production may be more complex than originally proposed. The pathway that generates electrons for hydrogen (fig. S12) is composed of many proteins encoded by multiple genes (tables S22 to S24). Our analyses extend the evidence that *T. vaginalis* hydrogenosomes contain the complete machinery required for mitochondria-like intraorganellar FeS cluster formation (*33*) and also reveal the presence of two putative cytosolic auxiliary proteins, indicating that hydrogenosomes may be involved in biogenesis of cytosolic FeS proteins. Some components of

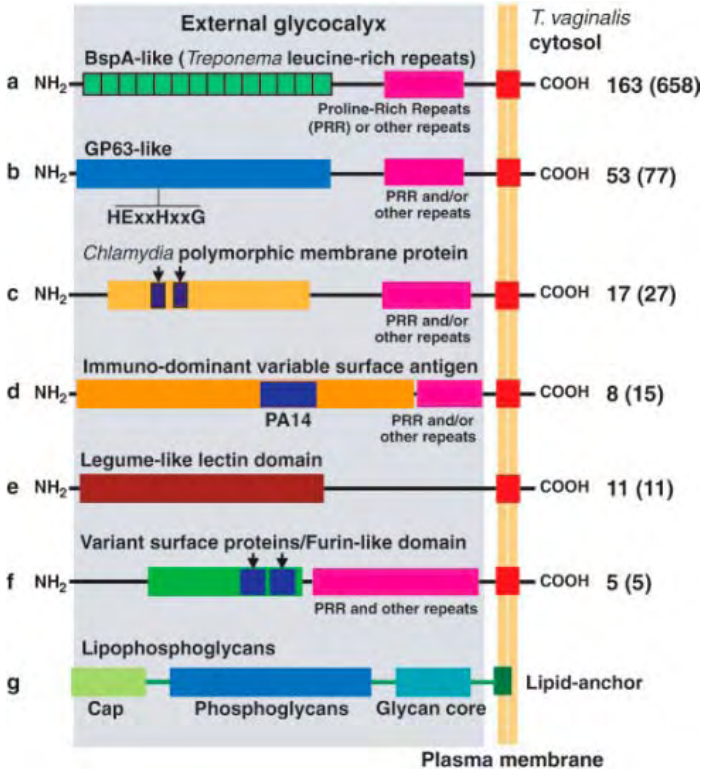

Fig. 3. Structural organization of putative *T. vaginalis* surface molecules involved in host cell adherence and cytotoxicity (*28*). Candidate surface protein families (a to f) are depicted as part of the glycocalyx (gray shading), known to be composed of LPG (g). The number of proteins with an inferred transmembrane domain (red box) is indicated at right, with the size of the entire family shown in parentheses. Substantial length variation exists between and within families (proteins are not drawn to scale). Additional information can be found in table S18.

FeS cluster assembly machinery have also been found in mitosomes (mitochondrial remnants) of *G. lamblia*, supporting a common evolutionary origin of mitochondria, hydrogenosomes, and mitosomes (*3*).

A new predicted function of hydrogenosomes revealed by the genome sequence is amino acid metabolism. We identified two components of the glycine-cleavage complex (GCV), L protein and H protein. Another component of this pathway is serine hydroxymethyl transferase (SHMT), which in eukaryotes exists as both cytosolic and mitochondrial isoforms. A single gene coding for SHMT of the mitochondrial type with a putative N-terminal hydrogenosomal presequence was identified. Because both GCV and SHMT require folate (fig. S12), which *T. vaginalis* apparently lacks, the functionality of these proteins remains unclear.

The 5-nitroimidazole drugs metronidazole (Mz) and tinidazole are the only approved drugs for treatment of trichomoniasis. These prodrugs are converted within the hydrogenosome to toxic nitroradicals via reduction by ferredoxin (Fdx) (fig. S12). Clinical resistance to Mz (Mz^R) is estimated at 2.5 to 5% of reported cases and rising (*34*) and is associated with decreases in or loss of Fdx (*35*). We identified seven Fdx genes with hydrogenosomal targeting signals (tables S21 and S25), the redundancy of which provides explanations for the low frequency of Mz^R and for why knockout of a single Fdx gene does not lead to Mz^R (*35*). Our analyses also provide clues to the potential mechanisms that clinically resistant parasites may use, such as the presence of nitroreductase (*NimA*-like), reduced nicotinamide adenine dinucleotide phosphate (NADPH)–nitroreductase, and NADH-flavin oxidoreductase genes (tables S23 and S26), which have been implicated in Mz^R in bacteria (*36*, *37*).

Summary and concluding remarks. Our investigation of the *T. vaginalis* genome sequence provides a new perspective for studying the biology of an organism that continues to be ignored as a public health issue despite the high number of trichomoniasis cases worldwide. The discovery of previously unknown metabolic pathways, the elucidation of pathogenic mechanisms, and the identification of candidate surface proteins likely involved in facilitating invasion of human mucosal surfaces provide potential leads for the development of new therapies and novel methods for diagnosis.

The analysis presented here of one of the most repetitive genomes known has undoubtedly been hampered by the sheer number of highly similar repeats and transposable elements. Why did this genome expand so dramatically in size? We hypothesize that the most recent common ancestor of *T. vaginalis* underwent a population bottleneck during its transition from an enteric environment (the habitat of most trichomonads) to the urogenital tract. During this time, the decreased effectiveness of selection resulted in repeat accumulation and differential gene family expansion. Genome size and cell volume are positively correlated (*38*, *39*); hence, the increased genome size of *T. vaginalis* achieved through rapid fixation of repeat copies could have ultimately resulted in a larger cell size. *T. vaginalis* cell volume is greater than that of *T. tenax* and related intestinal species *Pentratrichomonas hominis* (*40*) and *T. gallinae* (*41*), and it generally conforms to the relationship of genome size to cell volume reported for protists (*41*). *T. vaginalis* is also a highly predatory parasite that phagocytoses bacteria, vaginal epithelial cells, and host erythrocytes (*42*) and is itself ingested by macrophages. Given these interactions, it is tempting to speculate that an increase in cell size could have been selected for in order to augment the parasite's phagocytosis of bacteria, to reduce its own phagocytosis by host cells, and to increase the surface area for colonization of vaginal mucosa.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5809/207/DC1
Materials and Methods
SOM Text
Figs. S1 to S14
Tables S1 to S26
References
Data

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REPORTS

Spectropolarimetric Diagnostics of Thermonuclear Supernova Explosions

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Even at extragalactic distances, the shape of supernova ejecta can be effectively diagnosed by spectropolarimetry. We present results for 17 type Ia supernovae that allow a statistical study of the correlation among the geometric structures and other observable parameters of type Ia supernovae. These observations suggest that type Ia supernova ejecta typically consist of a smooth, central, iron-rich core and an outer layer with chemical asymmetries. The degree of this peripheral asphericity is correlated with the light-curve decline rate of type Ia supernovae. These results lend strong support to delayed-detonation models of type Ia supernovae.

Different supernova (SN) explosion mechanisms may lead to differently structured ejecta. Type Ia supernovae (SNe) have been used as premier tools for precision cosmology. They occur when a carbon and oxygen white dwarf reaches the Chandrasekhar stability limit, probably because of mass accretion in a binary system, and is disrupted in a thermonuclear explosion (*1*). Decade-long debates have discussed how the explosive nuclear burning is triggered and how it propagates through the progenitor star (*2*–*6*). Successful models generally start with a phase of subsonic nuclear burning, or deflagration, but theorists disagree on whether the burning front becomes supersonic after the earlier phase of deflagration. An explosion that does turn into supersonic burning is called delayed detonation (*4*). The resulting chemical structures are dramatically different for deflagration (*5*) and delayed-detonation models (*7*). For a pure deflagration model, chemical clumps are expected to be present at all velocity layers that burning has reached (*5*). For delayed detonation, the detonation front propagates through the ashes left behind by deflagration, burns partially burned or unburned elements further into heavier elements, and erases the chemically clumpy structures generated by deflagration.

The polarized emission from a supernova is caused by electron scattering in its ejecta. It is sensitive to the geometric structure of the ejecta (*8*). Electron scattering in an asymmetric ejecta would produce nonzero degrees of polarization (*8*). In continuum light, normal SNe Ia are only polarized up to about 0.3%, but polarization as high as 2% is found across some spectral lines (*9*–*14*). The polarization decreases with time and vanishes around 2 weeks past optical maximum. The low level of continuum polarization implies that the SN Ia photospheres are, in general, almost spherical. The decrease of polarization at later times suggests that the outermost zones of the ejecta are more aspherical than the inner zones. Similarly, the large polarization across certain spectral lines implies that the layers above the photosphere are highly aspheric and most likely chemically clumpy (*12*–*15*).

In this study, we report polarimetry of 17 SNe Ia. These are all the SNe Ia for which we have premaximum polarimetry. The observations (Table 1) were collected with the 2.1-m Otto Struve Telescope of the McDonald Observatory of the University of Texas and with one of the 8.2-m

Table 1. Identification, observing date, phase (in days relative to optical maximum light), telescope (McD indicates McDonald Observatory), intrinsic degree of polarization across the Si II 635.5-nm line, Δm_{15}, and references for Δm_{15} values. Numbers in parentheses indicate 1-σ error multiplied by 100.

SN	Date of observation	Phase	Telescope	P_{SiII}	Δm_{15}	References
1996X	14 April 1996	−4.2	McD 2.1 m	0.50(20)	1.30(02)	(*19*, *21*)
1997bp	7 April 1997	−5.0	McD 2.7 m	0.90(10)	1.29(08)	(*19*, *22*)
1997bq	25 April 1997	−3.0	McD 2.1 m	0.40(20)	1.17(02)	(*19*, *22*)
1997br	20 April 1997	−2.0	McD 2.1 m	0.20(20)	1.09(02)	(*19*, *23*)
1999by	9 May 1999	−2.5	McD 2.1 m	0.40(10)	1.79(01)	(*24*)
2001V	25 February 2001	−7.3	VLT	0.00(07)	0.73(03)	(*19*, *25*)
2001el	26 September 2001	−4.2	VLT	0.45(02)	1.16(01)	(*19*, *26*)
2002bo	18 March 2002	−5.0	VLT	0.90(05)	1.34(03)	(*19*, *27*)
2002el	14 August 2002	−6.4	VLT	0.72(09)	1.38(05)	(*19*)
2002fk	6 October 2002	−5.5	VLT	0.67(10)	1.19(05)	see text
2003W	31 January 2003	−4.5	VLT	0.64(10)	1.30(05)	see text
2004dt	13 August 2004	−7.3	VLT	1.60(10)	1.21(05)	(*28*)
2004ef	11 September 2004	−4.1	VLT	1.10(30)	1.54(07)	see text
2004eo	20 September 2004	−5.9	VLT	0.71(08)	1.46(08)	(*28*)
2005cf	1 June 2005	−9.9	VLT	0.44(05)	1.12(06)	(*28*)
2005de	6 August 2005	−4.4	VLT	0.67(14)	1.41(06)	see text
2005df	8 August 2005	−4.3	VLT	0.73(05)	1.29(09)	see text

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unit telescopes of the Very Large Telescope (VLT) of the European Southern Observatory (ESO). The typical spectral resolution of these observations is 1.2 nm, and the wavelength range is typically from 400 nm to 850 nm. The total exposure time was longer than 4 hours for data taken with the 2.1-m telescope and was around 1 to 2 hours for the ESO VLT data. Only SNe observed before optical maximum were included. We restricted ourselves to the characteristic Si II 635.5-nm line [Supporting Online Material (SOM) Text].

The observed Stokes parameters can be projected onto the so-called principal and secondary axes, which are defined (*13*, *16*) by a principal components analysis of the data points on the *Q*-*U* diagram such that the spectral variation of the polarization is maximal along the principal axis. The secondary axis is orthogonal to the principal axis. As an example, in the spectra and principal components of the polarization of SN 2002bo at different epochs (Fig. 1), the polarized spectral features at 470.0 nm and at 600.0 nm decreased significantly by day +14. The polarization data for SN 1996X, SN 1999by, SN 2001el, and SN 2004dt have been discussed in previous studies (*10*–*15*), revealing a considerable level of individuality.

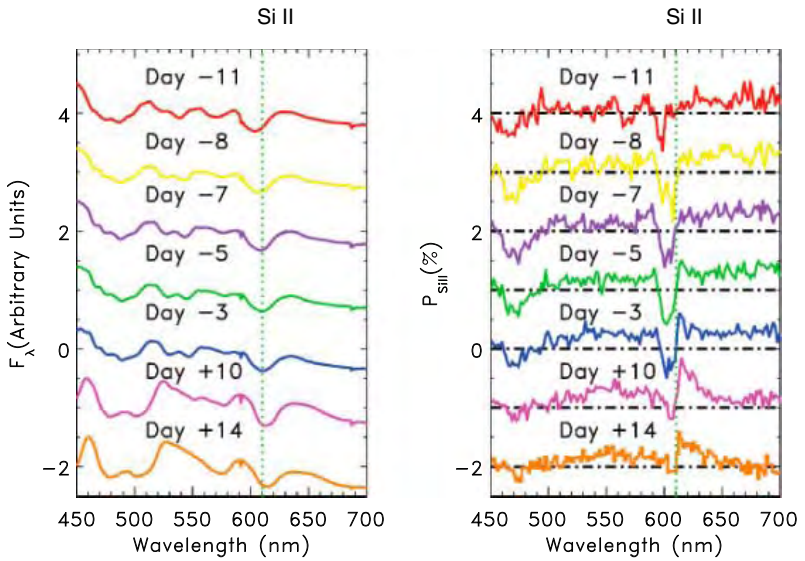

Fig. 1. Spectroscopy (**left**) and spectropolarimetry (**right**) of SN 2002bo. The units of the fluxes on the left are arbitrary; from top to bottom, the zero point of each spectrum is offset from zero by 4, 3, 2, 1, 0, −1, and −2 for clarity. On the right, from top to bottom, the degree of polarization of each observation is offset by 4%, 3%, 2%, 1%, 0%, −1%, and −2%. The vertical dotted lines mark the absorption features of the Si II lines.

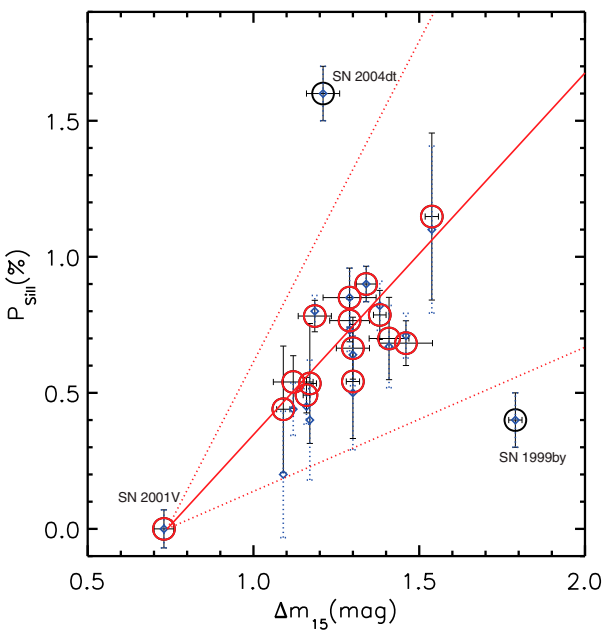

Fig. 2. The correlation between the degree of polarization across the Si II 635.5-nm line and the light curve decline rate, Δm_{15}, for a sample of 17 type Ia SNe. The blue diamonds are the measured values as given in Table 1. The open circles show the data corrected to day −5 by subtracting $0.041(t + 5) + 0.013(t + 5)^2$ from the observed degree of polarization, where t is the day after optical maximum. This correction formula was derived from a weighted quadratic fit to the time dependence of polarization (fig. S1). The linear fit represented by the straight line includes only spectroscopically normal SNe shown as red open circles. The blue open circle shows the highly polarized event SN 2004dt and the subluminous SN 1999by. The dotted lines illustrate the $1 - \sigma$ level of the intrinsic polarization distribution around the most likely value for the Monte Carlo simulation. Error bars around the data indicate 1-σ measurement errors.

As a luminosity indicator, we used the decline in *B* magnitude within 15 days from maximum (Δm_{15}). This quantity is found to be well correlated with the intrinsic luminosity of SNe Ia (*17*): Intrinsically dimmer SNe Ia usually show a faster decline, that is, larger Δm_{15}. For those SNe with no published light curves, the luminosity indicator was derived from cross-correlations of the flux spectra with a library of well-observed SNe Ia, and the Δm_{15} values of the closest spectral matches were adopted. The best matches to SN 2002fk, SN 2003W, SN 2004ef, SN 2005de, and SN 2005df are SN 1996X, SN 2002bo, SN 2001el, SN 2001el, and SN 1994D, respectively. The results were cross-checked with the ratio of the Si II absorption lines at 615 nm and 580 nm (*18*), and the results are generally in good agreement. For the SNe with no published light curves, 0.05 magnitude was quadratically added to the errors of Δm_{15} deduced from the light curve of the best matching spectral template. All Δm_{15} values derived from published light curves are based on our light-curve fitting procedure (*19*).

The degree of polarization is known to evolve with time. To compare the data at the same epoch, we fitted the time dependence of the polarization of all the Branch normal SNe by a second-order polynomial (fig. S1), and the polarization data were then corrected to 5 days before peak *B* magnitude. Excluding peculiar events SN 2001V, SN 2004dt, and SN 1999by (SOM Text), the correlation is fitted by a linear relationship, $P_{SiII} = 0.48(03) + 1.33(15)(\Delta m_{15} - 1.1)$. The Pearson correlation coefficient is 0.87, which suggests a strong correlation. The χ^2 of the linear fit is 16 with 13 degrees of freedom.

Because the continuum polarization is generally low (~0.2) for SNe Ia, the ejecta is likely to be chemically clumpy to explain the observed high polarization across spectral lines (*14*). If this is the case, projection effects must play a role in diluting this correlation (Fig. 2). This might be the case for the significant deviation of SN 2004dt from the regression line (Fig. 2). When viewed in certain directions, the degree of polarization can be particularly large or small. A tight correlation could only be expected for the mean degree of polarization averaged over several SNe and at the same photometric phase. To quantify this effect, we have constructed a toy model that assumed that the disk of the SN photosphere is polarized at 11.73% (*20*) at the limb and decreases quadratically with distance from the limb to the center to zero polarization (SOM Text). We performed a Monte Carlo simulation that assumes N clumps, each covering an area S along the line of sight to the photosphere. The depth of the P-Cygni absorption feature (defined as the ratio of flux at the minimum of the absorption feature to that of the continuum) is then $1 - NS/\pi R^2$, where R is the radius of the photosphere. For lines about 50% deep ($NS/\pi R^2 = 0.5$), the probability distribution of the polarization is found to peak at around 0.5% for $N = 20$. The $1 - \sigma$ width of this dis-

tribution, calculated assuming $NS/\pi R^2 = 0.5$ (Fig. 2), envelopes most of the observed data points. In reality, there is a wide range of Si II line strengths; accordingly, the numbers and sizes of lumps may not be the same for all the SNe. The observed polarization-Δm_{15} correlation appears to be tighter than that given by the Monte Carlo model. This is perhaps an indication of a non-negligible amount of large-scale asymmetry of the SN ejecta, especially for those SNe with high polarization and Δm_{15}. Such large-scale asymmetries do not generate noticeable amounts of polarization in the continuum, which is formed deeper inside, and can be identified with large plumes located above the SN photosphere. It may also be generated from the interaction of the ejecta with circumstellar material, such as an accretion disk before the explosion of the white dwarf progenitor. Alternatively, the observed tight correlation may be due to a global aspherical explosion. In this case, a tight correlation implies that a more asymmetric explosion generates intrinsically dimmer SNe. However, we stress that the asymmetries we observe here are confined to the high-velocity regions and do not affect the geometric shape of SN photosphere around the optical maximum. Any large-scale asymmetry is therefore confined only to the outermost layers.

Because the light curve of a SN Ia is powered by the radioactive decay of ^{56}Ni, we infer (Fig. 2) a possible anticorrelation between the amount of ^{56}Ni synthesized in SNe Ia and the asphericity of the silicon-rich layer.

Our result puts strong constraints on any successful models of SNe Ia. At around optical maximum, the photosphere is typically located at velocities around 12,000 km/s as measured from P-cygni line profiles, which according to hydrodynamic calculations (*7*) of delayed detonation is close to the velocity zones dominated by iron group elements. The absence of notable polarization at this velocity is evidence in support of delayed detonation.

Details of delayed-detonation models affect the brightness and the geometric structure of SNe Ia. Larger departures from sphericity imply that less of the central region is scoured of irregularities in the composition left by pure deflagration models, thus less material burned to thermonuclear equilibrium, and hence dimmer SNe, in accordance with the statistical trend revealed by our studies.

Lastly, we remark on the use of SNe Ia as standard candles. Asymmetry introduces intrinsic magnitude and color dispersions. Intrinsic color dispersion may be particularly important because it makes precise extinction corrections difficult. The stochastic nature of the origin of the asymmetry suggest that the color corrections can only be performed in a statistical sense. It is perhaps difficult to find pairs of SNe Ia with identical light curves and spectroscopic properties.

The application of spectropolarimetric observing techniques to SNe Ia permits the geometric structures of SNe to be probed even though they are at distances that cannot be spatially resolved. The explosion of SNe Ia is intrinsically a three-dimensional phenomenon, and a phase of delayed detonation is necessary to account for the observed geometric and chemical differentiation.

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Formation of a Nematic Fluid at High Fields in $Sr_3Ru_2O_7$

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In principle, a complex assembly of strongly interacting electrons can self-organize into a wide variety of collective states, but relatively few such states have been identified in practice. We report that, in the close vicinity of a metamagnetic quantum critical point, high-purity strontium ruthenate $Sr_3Ru_2O_7$ possesses a large magnetoresistive anisotropy, consistent with the existence of an electronic nematic fluid. We discuss a striking phenomenological similarity between our observations and those made in high-purity two-dimensional electron fluids in gallium arsenide devices.

In the standard materials that form the basis of most of today's electronic technology, the Hamiltonian for the outer electrons is dominated by the attraction to the ions of the crystalline lattice. In "strongly correlated" materials, this is no longer true. The Coulomb interaction between electrons is large, so it might be expected to add a large term to the Hamiltonian that is not necessarily strongly related to the periodic potential. However, in most correlated electron systems studied to date, the many-electron collective states still retain strong links with the lattice, and the range of "correlated electron matter" identified so far is considerably less diverse than

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should, in principle, be possible. In itinerant systems, it almost always consists of electron liquids such as Fermi liquids or superfluids that respect the lattice symmetry; the identification of superconductors in which the condensate breaks some lattice symmetries has been one of the triumphs of the field. [For a recent review, see (*1*).]

In recent years, there have been proposals that even more exotic electronic liquids might be observable. In an analogy with the nematic state of liquid crystals, which is characterized by orientational but not positional order, it might be possible to form nematic liquids in electronic systems with strong correlations (*2*). In the broadest sense, a correlated electron nematic is characterized by a lowering of rotational symmetry in its itinerant properties that is not simply a consequence of a symmetry lowering of the lattice.

We report electrical transport phenomena that show that the correlated electron system possesses this key property of a nematic fluid. In previous work, we have argued that a novel quantum phase forms in the vicinity of a metamagnetic quantum critical point in the correlated electron oxide $Sr_3Ru_2O_7$ (*3*–*5*). Here, we show that this state is accompanied by pronounced magnetoresistive anisotropies that have two-fold symmetry and can be aligned using modest in-plane magnetic fields. Even in the presence of these two-fold anisotropies, neutron single-crystal diffraction resolves no change from the initial square symmetry of the lattice. The overall phenomenology of our observations bears a striking resemblance to that observed in gallium arsenide (GaAs) devices near the high-field limit (*6*–*9*), which suggests that the nematic phenomena previously thought to be specific to close proximity to a fractional quantum Hall state may be more general.

The single crystals used in the present work were grown in an image furnace with techniques described fully in (*10*). All transport data shown are measurements of the in-plane magnetoresistivity, denoted ρ (*11*). Crystal purity is crucial in $Sr_3Ru_2O_7$. For a residual resistivity $\rho_o \approx 3$ μΩ·cm (corresponding to a mean free path of ~300 Å or less), the phase diagram contains a quantum critical point (QCP) that can be accessed by the application of a magnetic field of ~7.8 T parallel to the crystalline *c* axis and the effects of which have now been studied with a variety of experimental probes (*12*–*17*). As the purity is increased, first-order phase transitions appear as the QCP is approached, and measurements of at least five thermodynamic and transport properties contain features whose loci enclose a well-defined region of the phase diagram in the vicinity of the QCP. Previously, we have argued that these observations are indicative of the formation of a new quantum phase (*3*–*5*). For fields applied parallel to *c*, ρ has a pronounced anomaly when this phase is entered (Fig. 1A). The two steep "sidewalls" coincide with first-order phase transitions that can be observed using ac susceptibility or magnetization. The angle, θ, of the applied magnetic field to the *ab* plane of the crystal is a known tuning parameter in $Sr_3Ru_2O_7$ (*14*). Previous work has shown that the large resistive anomaly of Fig. 1A disappears rapidly with tilt angle (*18*), leaving behind much weaker signals in ρ that trace the origin of the two first-order phase transitions as a bifurcation from a single first-order transition at $\theta \approx 60°$ (*5*), marked by the white arrow in Fig. 2A. At first sight, this seems to contradict the identification of the bounded region between the two first-order lines as a single distinctive phase; there was no evidence that the sudden drop in resistivity with the angle at $\theta \approx 80°$ coincided with any phase boundary. The apparent contradiction can be resolved by postulating the existence of domains of some kind. In such a picture, the behavior shown in Figs. 1A and 2A would be due to these domains producing the extra scattering. The fact that the anomalous scattering disappears so rapidly as θ increases would then be most straightforwardly interpreted as being due to the in-plane component of the tilted magnetic field ($H_{in\text{-}plane}$) destroying the domains.

However, instead of simply removing the anomalous peak, $H_{in\text{-}plane}$ exposed an intrinsic asymmetry of the underlying phase, defining "easy" and "hard" directions for magnetotransport. These easy and hard directions are shown in Fig. 1B. In previous experiments (*3*–*5*), we had worked with the current $I \parallel b \perp H_{in\text{-}plane}$, so that the standard metallic transverse magnetoresistance ρ_{bb} could be studied across the whole phase diagram. In that configuration (red traces in Fig. 1), the anomalous scattering disappears rapidly as $H_{in\text{-}plane}$ increases. However, the behavior of ρ_{aa} (measured with $I \parallel a \parallel H_{in\text{-}plane}$) is completely different. As shown by the black traces in Fig. 1, the scattering rate remains high even for an angle at which the anomalous scattering is absent for $I \perp H_{in\text{-}plane}$.

Pronounced in-plane resistive anisotropy can have a number of origins. There is known to be a strong magnetostructural coupling in $Sr_3Ru_2O_7$, so one possibility is a symmetry-lowering structural phase transition giving the resistive anisotropy due to a corresponding anisotropy in the hopping integrals. Another is the formation of field-alignable magnetic domains such as those examined in the context of itinerant metamagnetism in recent theoretical work (*19*, *20*).

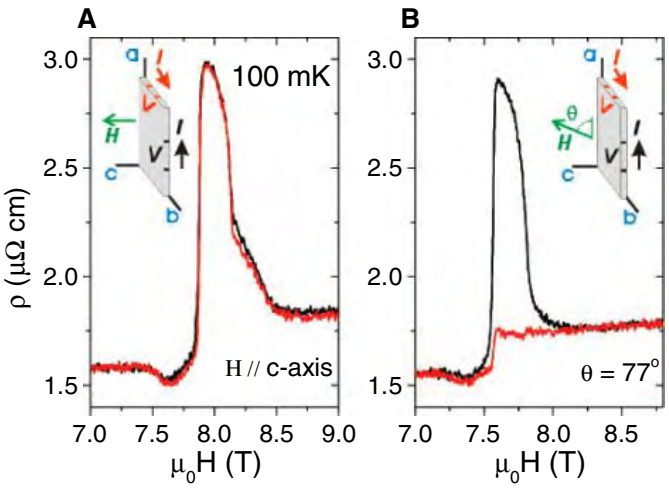

Fig. 1. The two diagonal components ρ_{aa} and ρ_{bb} of the in-plane magnetoresistivity tensor of a high-purity single crystal of $Sr_3Ru_2O_7$. (**A**) For an applied field parallel to the crystalline *c* axis (with an alignment accuracy of better than 2°), ρ_{aa} (black) and ρ_{bb} (red) are almost identical. (**B**) With the crystal tilted such that the field is 13° from *c*, giving an in-plane component along *a*, a pronounced anisotropy is seen, with the easy direction for current flow being along *b*, perpendicular to the in-plane field component (*18*). If the in-plane field component is aligned along *b* instead, the easy direction switches to current flow along *a*.

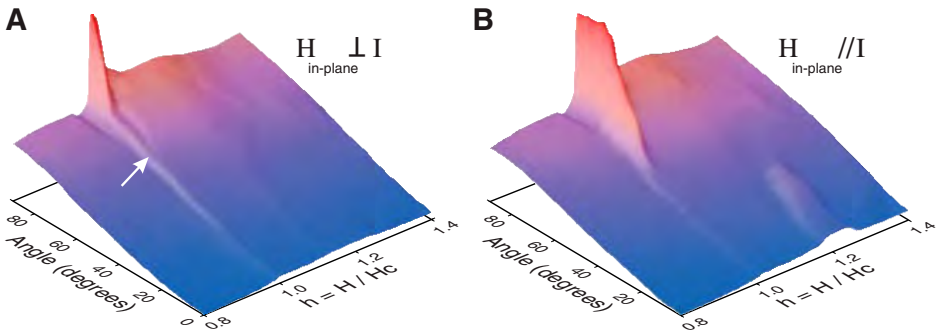

Fig. 2. Three-dimensional plots of the magnetoresistivity components ρ_{bb} (**A**) and ρ_{aa} (**B**) of a single crystal of $Sr_3Ru_2O_7$ as the external magnetic field is rotated from alignment along the crystalline *a* axis (0°) to alignment along the crystalline *c* axis (90°), at a constant temperature of 100 mK. The quantity $H_c(\theta)$ that normalizes *h* is the main metamagnetic transition (i.e., the one that dominates the change in the magnetic moment). It varies smoothly from 5.1 T at 0° to 7.87 T at 90°. The same data plotted without this normalization are shown in SOM Text 3.

To investigate these possibilities, we first carried out measurements of ρ(**H**) and magnetic susceptibility χ(**H**) at temperatures between 20 mK and 4 K on 20 samples from three different batches with a wide variety of shapes, 6 of which were cut from the same piece of crystal (SOM Text 3). Shapes vary from square plates with sides $A \approx B >> C$ to rectangular plates with side $A >> B >> C$ to long cylinders with $A \approx B << C$. In each class of sample, dimension C is aligned with the crystalline c axis, but we deliberately tested combinations of A and B that were aligned or misaligned with the crystalline a and b axes (SOM Text 1). An important result of all these experiments is that, apart from a small region of hysteresis (maximum width ~80 mT), all the first-order phase boundaries observed in $Sr_3Ru_2O_7$ were invariant under changes to the sample shape. This firmly rules out demagnetization effects as playing a major role in determining the physics and, hence, directly contradicts predictions concerning magnetic domains (*20*). To check for a large spontaneous lattice parameter anisotropy, we performed elastic neutron scattering measurements for $H \,//\, c$ (SOM Text 2). Within our experimental resolution of 4×10^{-5} Å, we see no evidence of any difference in lattice parameters a and b in the anomalous region.

The experiments described above therefore rule out two of the more standard explanations (magnetic domains and structural change) for the anisotropy shown in Fig. 1B. In Fig. 2, we show the complete magnetoresistive field-angle "phase diagrams" for fields between 4.2 T and 9 T rotating the entire 90° from parallel to a to parallel to c (θ = 90°). The temperature is 100 mK. Figure 2A shows the magnetoresistivity for the easy direction, ρ_{bb}, and Fig. 2B that for the hard direction, ρ_{aa}.

Figure 3 shows typical data for the difference in ρ between the hard and easy directions (main figure) and the temperature dependence of ρ in a field of 7.4 T along both directions (upper inset). Along the easy direction, standard metallic behavior is seen, whereas a nonmetallic temperature dependence is seen for the hard direction [in agreement with results reported previously (*3*, *4*)]. In the presence of such temperature-dependent anisotropy, it is reasonable to plot the difference between ρ_{aa} and ρ_{bb}, normalized by their sum, as a phenomenological order parameter. This is done in the lower inset to Fig. 3 at a field applied at θ = 72°.

Several striking features are evident from our data: (i) A key finding is that strong scattering can be observed in the whole region of the phase diagram enclosed by the two first-order phase boundaries that bifurcate from a single first-order transition line at θ ≈ 60° (*5*). If there is an in-plane field component, this scattering becomes strongly anisotropic. The present observations are important because if one interprets the lower inset of Fig. 3 as evidence for an order parameter, one sees that the phase that it describes is bounded (at low temperatures and constant θ) by well-defined first-order phase transitions that are independent of extrinsic parameters such as sample shape. (ii) Although the easy direction in Fig. 2 is for currents passed along the b axis, this is determined by the in-plane field component having been directed along a for the data shown. If the direction of the field is rotated by 90°, the anisotropy is reversed and a becomes the easy direction. Checks show that the rotation is not smooth; the easy direction is either along a or b but cannot be made to lie, for example, at 45°. (iii) As can be seen in the lower inset to Fig. 3, the presence of a (small) symmetry-breaking $H_{in\text{-}plane}$ slightly rounds off the transition in temperature, giving a "tail" above 800 mK. This effect, which highlights the fact that an in-plane field (that breaks rotational symmetry) is conjugate to the order parameter, becomes more pronounced for lower angles, that is, for higher $H_{in\text{-}plane}$. (iv) Another feature, weaker, broader, but still very noticeable, is seen in the hard direction for θ < 40° and $h \approx 1.2$ (Fig. 2B). (v) The anisotropy described in point (iv) is, like the one described in point (i), extremely sensitive to sample purity [(*21*) and SOM Text 4], strongly suggesting a common origin for the two. Its breadth in field and in temperature (not shown) is also consistent with its proximity to the *ab* plane, that is, in the presence of a large in-plane magnetic field. Specific heat data taken cooling down at its central field ($h \approx 1.2$) show a logarithmic divergence of C/T down to 1 K (*21*), giving good evidence that this feature, like that for fields parallel to c, is related to incipient quantum criticality.

The combination of susceptibility, neutron scattering, and transport data gives strong evidence for the spontaneous formation of a structured, anisotropic state in the correlated electron fluid as a quantum critical point is approached in $Sr_3Ru_2O_7$. Although the anisotropy is seen explicitly in the presence of a weak symmetry-breaking in-plane field component, there is compelling evidence that the symmetry breaking of the correlated electron state is spontaneous and exists even for $H \,//\, c$. The data shown in Fig. 1A can be reconciled with those at other parts of the phase diagram (Fig. 1B and Fig. 2) if the hard axis is randomly oriented along the a and b directions in different regions of the sample, leading to overall isotropy despite strong local anisotropy. Such behavior is commonplace in symmetry-broken states, for example, in simple ferromagnets in zero applied magnetic field (*22*).

Interesting comparisons can be made between the present data and those in other correlated systems. In-plane transport anisotropies have been observed in both cuprates and manganites. In those systems, the crystals are always orthorhombic, but in some cases the anisotropy increases while the degree of orthorhombicity decreases, and strong anomalies have been seen in the Hall effect, both of which have been interpreted as evidence for spontaneous charge-stripe formation (*23*–*25*). A much stronger similarity exists between our observations and those on two-dimensional (2D) GaAs devices. For example, it was shown (*6*–*9*) that if the devices can be prepared with ultrahigh mobility such that the fractional quantum Hall effect (FQHE) could be observed in the upper two Landau levels, the correlated electron system does not make a simple FQHE-Fermi liquid crossover as the field

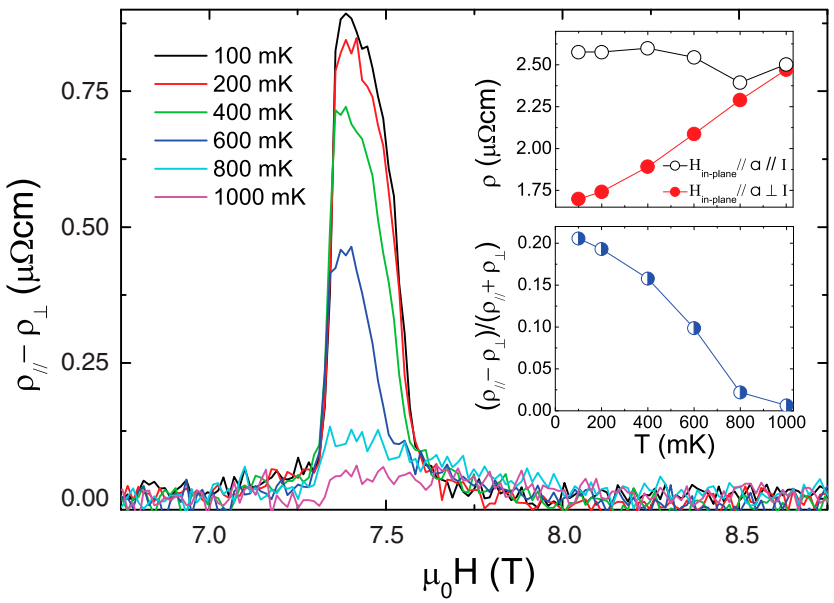

Fig. 3. The temperature dependence of the difference between ρ_{aa} and ρ_{bb} for fields applied at θ = 72° such that the in-plane field component lies along a. (**Upper inset**) The temperature dependence of ρ_{aa} (black) and ρ_{bb} (red) for $\mu_o H = 7.4$ T applied in the direction specified above. For this field orientation, ρ_{bb} has a clearly metallic temperature dependence, whereas ρ_{aa} has the mildly nonmetallic temperature dependence previously reported for $H \,//\, c$ in (*3*, *4*). If the in-plane field component is instead oriented along b, ρ_{bb} and ρ_{bb} switch in both magnitude and temperature dependence. (**Lower inset**) The temperature dependence of the difference between the two magnetoresistivities shown in the upper inset, normalized by their sum, which is similar to that expected for the order parameter associated with a continuous thermal phase transition.

is reduced and the filling is increased. In the $N = 2$ to $N = 5$ Landau levels, the FQHE is replaced by a strong spontaneous resistive anisotropy aligned with principal in-plane crystal axes, even though the crystal symmetry shows no evidence of orthorhombicity. The anisotropy exists even for fields perpendicular to the plane of the device (presumably because of some symmetry-breaking gradients introduced during device fabrication) but can be rotated by 90° by applying a modest in-plane field. Just as in the present observations on $Sr_3Ru_2O_7$, the GaAs data have strong temperature and purity dependencies, and the easy direction lies perpendicular to an in-plane field applied along one of the two relevant crystalline principal axes.

The phenomenological similarity between the GaAs and $Sr_3Ru_2O_7$ results suggests a common origin for the observations. The disorder dependence gives an important clue, because strong sensitivity to elastic scattering is the signature of a state that is anisotropic in **k**-space, as is well known in unconventional superconductivity (*26*). The challenge is how to reconcile what are, apparently, large differences in the starting physical situations. To promote self-organization of a correlated electron system, one must tune the ratio of a potential energy term often summarized by the parameter U (the Coulomb repulsion, which tends to localize) to the kinetic energy, often denoted by W, which tends to delocalize. If this is done "chemically," by forming new compounds, the change in the U/W ratio is strongly linked to a change to the electron-lattice coupling, because increasing U and decreasing W also involves increasing the strength of the periodic potential. In the GaAs devices, it is possible to increase the U/W ratio by quenching the kinetic energy by going to very low Landau levels. This leads to a relatively high effective correlation strength without an increase in the effective strength of the periodic potential. In $Sr_3Ru_2O_7$, similar basic physics is taking place but with a different kind of tuning. It is intrinsically a strongly correlated material, so the starting periodic potential is much larger than in GaAs. However, the existence of an underlying metamagnetic quantum critical point makes the quasiparticle mass m^* diverge on the approach to criticality (*15*, *27*). This mass divergence is another route to increasing U/W without increasing the strength of the periodic potential, hence freeing the correlated electron fluid from its rigid link to the underlying lattice.

We have shown that in highly restricted parts of its phase diagram, in proximity to metamagnetic quantum critical points, the electron fluid in $Sr_3Ru_2O_7$ develops a strong resistive anisotropy, whose hard and easy axes can be interchanged by the application of modest in-plane magnetic fields. The data are consistent with the formation of a nematic state with broken rotational symmetry. Intriguingly, a correlated electron nematic arising from a Pomeranchuk-like Fermi surface distortion (*4*, *28*–*36*) possesses two of the key features that are present in our data and those from GaAs, namely the **k**-space anisotropy that would give a strong disorder dependence and the possibility of anisotropic transport, intrinsic or through domain formation (*37*). Whatever the detailed microscopic origin (*38*), our data suggest that nematic behavior is a feature of ultraclean low-dimensional correlated electron systems in which the bandwidth can be reduced independently of changes to the strength of the periodic potential.

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Figs. S1 to S4

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Highly Siderophile Element Constraints on Accretion and Differentiation of the Earth-Moon System

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A new combined rhenium-osmium– and platinum-group element data set for basalts from the Moon establishes that the basalts have uniformly low abundances of highly siderophile elements. The data set indicates a lunar mantle with long-term, chondritic, highly siderophile element ratios, but with absolute abundances that are over 20 times lower than those in Earth's mantle. The results are consistent with silicate-metal equilibrium during a giant impact and core formation in both bodies, followed by post–core-formation late accretion that replenished their mantles with highly siderophile elements. The lunar mantle experienced late accretion that was similar in composition to that of Earth but volumetrically less than (~0.02% lunar mass) and terminated earlier than for Earth.

The histories of Earth and the Moon are intrinsically linked, with a catastrophic giant impact considered to be the most likely mode of origin of the Earth-Moon system (*1*, *2*). After this event, both bodies experienced early [4.53 ± 0.01 gigayear (Gyear)] global-scale differentiation (*3*, *4*), forming metallic cores and silicate mantles and crusts. However, uncertainty exists in

several aspects of these planetary evolution models. For example, the influence of core formation and the relative timing and composition of late-accretion material to the Moon's mantle from bodies striking the surface are poorly constrained (*5*, *6*). This has important consequences for metal-silicate equilibrium (*5*), the so-called late veneer on Earth (*7*) and its apparent relation to the rise of life (*8*), and lunar late heavy bombardment (*9*).

Highly siderophile elements (HSEs: Re, Au, Ir, Os, Ru, Rh, Pt, and Pd) and the Re-Os isotope system embedded within these elements offer a means to address these problems, because they are effective tracers of the early stages of planetary evolution (*10*). They are sensitive to metal-silicate equilibria during core formation and to the subsequent addition of primitive, HSE-rich undifferentiated materials to silicate mantles after core segregation. The abundances of HSEs in Earth's mantle (~0.008 × C1 chondrite) are elevated relative to those predicted from metal-silicate equilibrium (≤0.00001 × C1 chondrite). Although high-temperature, high-pressure silicate-metal equilibrium can account for depletion of HSEs in planetary mantles, it cannot explain the chondritic proportions of HSEs in Earth. This observation is considered to be primary evidence for late accretion after core formation (*6*, *7*). In contrast, great uncertainty exists regarding lunar-mantle HSE abundances (*5*). Determining lunar-mantle HSE contents has been problematic because of a lack of direct mantle samples, together with low HSE abundances in mare basalts, melts of the lunar interior, and potential mantle proxies. Severe leaching of pyroclastic glasses, which have experienced meteoritic and magmatic condensate contamination, and analysis of a dunite cumulate point to lower HSE abundances for the Moon's mantle as compared with Earth's, but more substantive data are needed to draw any conclusions (*11*).

We report precise Os-isotope– and HSE-abundance data (table S1) for five basalts from the Apollo 15 mission, six from Apollo 17, and six lunar basalts of meteoritic origin from LaPaz, Bolivia, that were obtained by using an ultra-low–blank, isotope-dilution digestion technique (*12*). In contrast to pyroclastic glasses, the selected samples are unaffected by meteoritic contamination (*13*–*15*) and hence offer an opportunity for determining the HSE and Os-isotope compositions of the lunar mantle.

A striking feature of the data is that lunar basalts have lower HSE abundances than terrestrial mid-ocean ridge basalts (MORBs) with comparable-to-lower MgO contents (Fig. 1). In some samples, Ir-Pt–group elements (I-PGEs: Os, Ir, and Ru) are fractionated to lower abundances than Pd-PGEs (P-PGEs: Pt and Pd), but others have relatively flat, unfractionated PGE patterns. Fractionation of I-PGE and P-PGE results from extensive partial melting or crystal/melt separation, in which more compatible I-PGEs remain in the mantle residue or are trapped within early-formed minerals (*16*, *17*). Variations in mare-basalt HSE abundances and PGE patterns can be explained by fractionation of olivine and chromite, as recognized from their major-element compositions (*18*, *19*). LaPaz basalts have large I-PGE and P-PGE fractionations relative to Apollo basalts (Fig. 1), consistent with their incompatible-element–enriched and –fractionated nature (*13*). Measured $^{187}Os/^{188}Os$ compositions for Apollo 15 and 17 basalts (0.1265 to 0.1729) are nearly chondritic as compared with the LaPaz basalts (0.1697 to 0.4628), in agreement with a preliminary study of two of the basalts (*20*). Initial $^{187}Os/^{188}Os$ ratios range from within 5% of chondritic values to values far below the solar system initial composition.

Another remarkable attribute of the data set is the ubiquitously low Re contents of lunar basalts, which were previously ascribed to low oxygen fugacity (fO_2) in the lunar mantle, affecting HSE partitioning during melting (*20*). However, postulated redox effects on Re compatibility during melting have not been experimentally verified and assume similar S contents for the lunar and terrestrial mantles (*20*). Studies of mare-basalt olivine melt inclusions indicate that the lunar mantle has S contents that are two to three times lower than in the terrestrial mantle, such that fS may be more dominant than fO_2 in controlling HSE partitioning (*21*). Our data demonstrate that Pt and Pd are also anomalously low in the lunar mantle, as are Ir, Ru, and Os. PGEs are unlikely to be as redox-sensitive as Re, and hence, our view is that fO_2 cannot be the dominant control on low lunar HSE abundances, even if it cannot be ruled out for controlling Re.

Samples with low initial $^{187}Os/^{188}Os$ have low MgO and Os abundances [<10 parts per trillion (ppt)], with measured $^{187}Os/^{188}Os$ being the most divergent from chondrites. PGE patterns for these basalts show the greatest interelement fractionation, with $(Pd/Ir)_n$ and $(Os/Ir)_n$ (where n denotes the chondrite-normalized ratio) deviating substantially from the chondritic value of 1. Re enrichments with $(Re/Os)_n > 1$ are also evident. The very low Re and Os abundances in these samples make them extremely susceptible to minor Re addition or Os loss [supporting online material (SOM) text]. Their initial $^{187}Os/^{188}Os$ ratios are too low for their sources to have interacted with a component with low time-integrated Re/Os before magmatism. Although we are not able to conclusively determine whether Re addition or Os loss is responsible, these basalts have obviously experienced a multistage evolution. We classified these basalts as "disturbed" and did not consider them in constraining the evolution of the lunar mantle.

Mare basalts with generally higher MgO contents have relatively elevated I-PGE contents

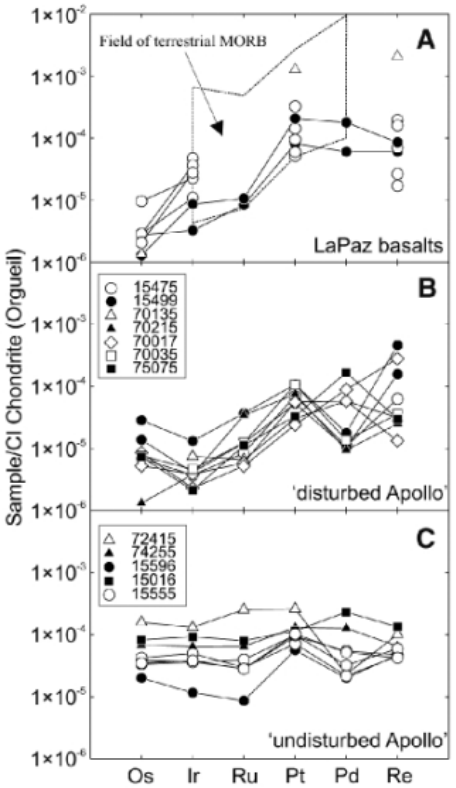

Fig. 1. Chondrite-normalized (Orgueil C1) PGE patterns for lunar mare basalts versus terrestrial MORBs. (**A**) LaPaz basalts, including CT (open circles), HPA digestions (black circles), and the fusion crust sample no. LAP 02224, 17 (open triangles). (**B** and **C**) Apollo 17 (high-Ti) and Apollo 15 (low-Ti) disturbed (B) and undisturbed (C) basalts (SOM text).

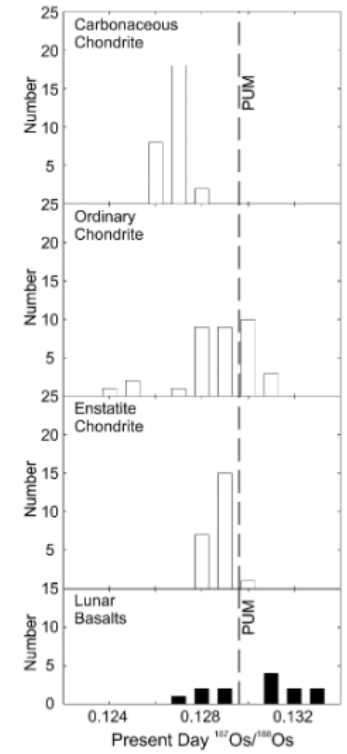

Fig. 2. Histogram range of the measured $^{187}Os/^{188}Os$ values for chondrites versus lunar basalts. The lowest measured $^{187}Os/^{188}Os$ values for mare basalts lie in the range of chondritic compositions, and the primitive upper mantle (PUM) composition is derived from terrestrial-mantle peridotites (SOM text). Number indicates the number of samples analyzed.

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(Os >10 ppt), unfractionated PGE patterns with $(Pd/Ir)_n$ and $(Os/Ir)_n$ close to 1, and initial Os isotopic compositions that are within 5% of chondritic values (Figs. 1 and 2 and table S1). We classified these samples as "undisturbed" (74255, 15016, 15555, and 15596) and used them to consider Os-isotope and HSE compositions of lunar-mantle source regions. Undisturbed samples have origins consistent with mantle-derived melts that have experienced olivine and spinel accumulation (*19*, *20*). The effect of this accumulation would be to increase I-PGE abundances (*17*), thereby flattening PGE patterns. The enhanced I-PGE abundances result in estimates of the probable conservative maxima of lunar-mantle HSE abundances. Near-chondritic Os-isotope ratios of undisturbed basalts indicate derivation from a source that experienced long-term chondritic evolution of Re/Os (and by inference, HSE ratios), which maintained this character through the eruptive duration of the basalts, until at least 1 Gyear after Moon formation.

It is unlikely that HSE ratios would remain close to chondritic values if they were affected by contamination from the lunar crust during the eruption of the basalts (SOM text). The selected samples have been well characterized and show no evidence of crustal or meteoritic contamination (*14*, *15*, *18*, *19*). Therefore, unfractionated PGE patterns among undisturbed mare basalts probably result from relatively small degrees of melting (<<15%, SOM text), plus minor olivine and chromite accumulation.

Extrapolations to mantle abundances from basaltic compositions are complicated by uncertainties in HSE mantle-melt partitioning (*5*). An empirical approach is to compare mantle-melt products from the Moon with those from Earth, assuming that relative offsets reflect the same magnitude of offset in mantle HSE contents (*11*). This comparison is most robustly done with I-PGEs that are least affected by degassing effects on Earth (*22*). HSE abundances were estimated by regressing lunar and terrestrial PGE data to an assumed fertile mantle MgO composition, accounting for the lower MgO

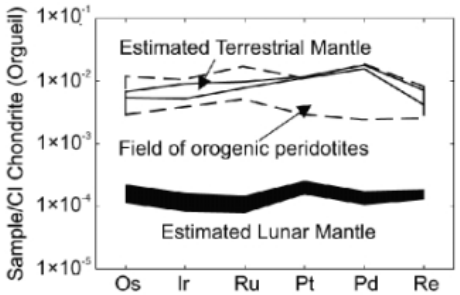

Fig. 3. Estimated lunar- and terrestrial-mantle PGE abundances and patterns versus terrestrial orogenic peridotites. Estimates for mantle compositions were made by regressing Os, Ir, Ru, Pt, Pd, and Re versus MgO data (SOM text and fig. S2). Both lunar and terrestrial mantles have chondritic relative abundances of HSEs, but the Moon's mantle possesses abundances nearly two orders of magnitude less than those of Earth. Calculated concentrations for the lunar mantle are ~0.2 ng/g Pt; 0.1 ng/g Os, Ir, Ru, and Pd; and 0.01 ng/g Re; ~0.0002 × C1 chondrite.

of the lunar mantle. Results yield an estimated terrestrial-mantle composition with nearly chondritic relative abundances (~0.008 × C1 chondrite) and a PGE pattern similar to that of fertile orogenic peridotites (Fig. 3). Regressed data for undisturbed lunar basalts also generate broadly chondritic relative abundances of HSEs, but with all HSE abundances 20 to 40 times lower than terrestrial-mantle estimates (~0.0002 × C1 chondrite, Fig. 3).

Three key observations that place important constraints on lunar evolution were predicted from our data set: (i) low HSE abundances, (ii) chondritic HSE proportions, and (iii) the long-term chondritic Os-isotopic composition of the Moon's mantle. Two of these characteristics [(ii) and (iii)] are also shared with Earth and point to similar processes being involved in the formation and evolution of Earth and the Moon. A two-stage evolution is required to explain these observations.

Abundances of HSEs were initially lowered in the lunar and terrestrial silicate mantles by metal-silicate partitioning. For both Earth and the Moon, scavenging of HSEs could have occurred through two processes. HSE-depleted mantles may have been inherited during the giant impact, where models predict that nearly all core material for both colliding bodies would have been sequestered into Earth (*1*, *2*). Alternatively, the segregation of cores during planetary differentiation would strip HSEs from silicate mantles. Metal-silicate equilibrium would fractionate HSE ratios, in particular, Re from Os; therefore, a second stage is required to replenish HSEs back to chondritic-relative proportions to ensure a chondritic Os-isotopic evolution for the lunar mantle.

For Earth, post–core-formation accretion of ~0.4% of the mantle mass is thought to have occurred, re-enriching the upper mantle in HSEs by effective mixing of material during mantle convection (*6*). The observed depletion in lunar-mantle HSEs indicates that the late accretion component was much less important for the Moon. We calculated that the addition of ~0.02% (1.5×10^{19} kg) of the Moon's mass by accretion of chondritic material would restore chondritic Re/Os and account for the inferred lunar-mantle HSE composition. This is equivalent to an Earth/Moon mass flux ratio of ~2700, which is considerably greater than the ratio of 30 that was estimated from the gravitational-attraction potential of the two bodies (*6*). Because the lunar cratering record indicates substantial continued impacts after crust formation, we suggest that the thick, ancient >4.4-Gyear (*23*) lunar crust played a key role in isolating the lunar mantle early in the late accretion phase of lunar evolution, explaining its limited HSE re-enrichment. The mixing and homogenization of any meteoritic post–core-formation HSE inventory to re-create a chondritic mantle for Re-Os isotopes probably occurred in the ~0.1-Gyear period between the inception of lunar differentiation [4.53 ± 0.01 Gyear (*3*)] and the stabilization of the lunar crust (*23*). This model, which explains the abundances and chondritic ratios of HSEs in lunar basalts, agrees with other physical and dynamical constraints on the formation and evolution of the Moon. Mare-basalt HSE data are, therefore, consistent with the giant impact and magma-ocean differentiation models for the Moon followed by prolonged, higher-volume late accretion that was responsible for elevated HSE abundances in Earth's mantle relative to the Moon.

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12. Samples were spiked with a multi-element tracer followed by Carius-tube (CT) or high-pressure–asher (HPA) digestion and solvent extraction for Os and anion exchange column chemistry for Ir, Ru, Pt, Pd, and Re. Long-term $^{187}Os/^{188}Os$ reproducibility using the University of Maryland, College Park, standard was 2.7 per mil for standard load sizes of 1.8 to 175 pg/g measured during the course of this study (0.11387 ± 0.00031, 2σ, n = 17, where n is the number of standards analyzed). Blanks for the CT method had the following element concentrations: 0.37 ± 0.05 pg/g [Os], 8 ± 1 pg/g [Re], 2.2 ± 0.1 pg/g [Ir], and 100 ± 10 pg/g [Pt]; $^{187}Os/^{188}Os$ = 0.1577 ± 0.0011. Blanks for the HPA method had the following element concentrations: 0.067 ± 9 pg/g [Os], 0.79 ± 0.08 pg/g [Re], 0.9 ± 1.0 pg/g [Ir], 0.82 ± 0.07 pg/g [Ru], 3.3 ± 2.3 pg/g [Pt], and 9.4 ± 9.0 pg/g [Pd]; $^{187}Os/^{188}Os$ = 0.290 ± 0.007. Materials and methods are available as supporting material on *Science* Online.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5809/217/DC1
Materials and Methods
SOM Text
Figs. S1 and S2
Table S1
References

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Stabilization of Platinum Oxygen-Reduction Electrocatalysts Using Gold Clusters

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We demonstrated that platinum (Pt) oxygen-reduction fuel-cell electrocatalysts can be stabilized against dissolution under potential cycling regimes (a continuing problem in vehicle applications) by modifying Pt nanoparticles with gold (Au) clusters. This behavior was observed under the oxidizing conditions of the O_2 reduction reaction and potential cycling between 0.6 and 1.1 volts in over 30,000 cycles. There were insignificant changes in the activity and surface area of Au-modified Pt over the course of cycling, in contrast to sizable losses observed with the pure Pt catalyst under the same conditions. In situ x-ray absorption near-edge spectroscopy and voltammetry data suggest that the Au clusters confer stability by raising the Pt oxidation potential.

After Haruta's discovery (*1*) of the catalytic activity of supported small Au clusters for CO oxidation, there were several reports on the effects of the oxide supports in facilitating this activity (*1*–*3*). Other explanations of the activity included the clusters' distinct electronic (*4*) or chemical properties (*5*). The mechanism of oxygen adsorption and activation necessary for rapid CO oxidation is controversial and opposite to the observed lack of O_2 dissociation on Au single crystals (*6*). Recently, Chen and Goodman (*7*) suggested that all reactants adsorb on Au rather than on the oxide support. The metal oxide/support interface boundary sites are believed to be important for stabilizing oxygen-containing reaction intermediates on Au clusters (*8*). Support effects were reported on the nucleation, growth, and morphology of Au nanoclusters for TiO_2 and SiO_2 oxides (*9*).

As the underlying surface affects the Au clusters, so the clusters can conversely be expected to alter the properties of the support surfaces. However, such effects have not yet been studied, despite considerable scientific and technological interest. Here we report that Au clusters have a stabilizing effect on an underlying Pt metal surface under highly oxidizing conditions and suppress Pt dissolution during the O_2 reduction reaction (ORR) during potential cycling, without decreasing the oxygen reduction kinetics.

Fuel cells are expected to become a major source of clean energy (*10*, *11*) with particularly important applications in transportation. Despite considerable recent advances, existing fuel-cell technology still has drawbacks, including the instability of the Pt electrocatalyst for the ORR at the cathode (*10*). Recent work recorded a substantial loss of the Pt surface area over time in proton-exchange membrane fuel cells (PEMFCs) (*11*) during the stop-and-go driving of an electric car; this depletion exceeded the Pt dissolution rates observed upon holding at constant potentials (*12*) for extended time spans. Our results show promise toward resolving this impediment.

The Au clusters were deposited on a Pt catalyst (carbon-supported Pt nanoparticles) through galvanic displacement by Au of a Cu monolayer on Pt (*13*). Underpotential deposition, which involves a monolayer-limited process at potentials above the thermodynamic values, was used to coat a Pt surface with a monolayer of Cu. To obtain some insights into a possible mechanism of Au cluster formation on Pt nanoparticles, we describe a more tractable model system: depositing Au onto a single-crystal Pt(111) substrate. In addition, the Pt(111) surface is known to form a surface oxide layer that inhibits ORR activity and leads to its possible dissolution. It is likely that Au clusters can affect this process, which can provide information on the stabilization effect. After undergoing several potential sweeps to 1.2 V, the Au monolayer transformed into three-dimensional clusters (Fig. 1). The scanning tunneling microscopy (STM) image shows clusters two to three monolayers thick and 2 to 3 nm in diameter. All potentials are given with respect to a reversible hydrogen electrode (RHE). The measurements were carried out at room temperature, unless otherwise indicated.

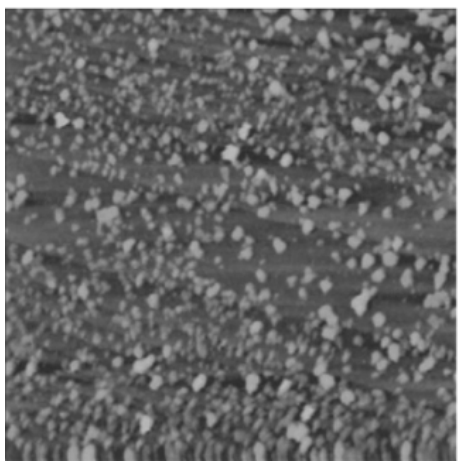

Fig. 1. STM image (125 × 125 nm) of the Au clusters on a Pt(111) surface, obtained by galvanic displacement of a Cu monolayer by Au. A Cu monolayer was deposited at underpotential on Pt (111). The Au adlayer was subjected to 10 cycles between 0.2 and 1.2 V versus RHE with a sweep rate of 50 mV/s to obtain such clusters. The STM image was acquired at the electrode potential of 0.8 V in 0.1 M $HClO_4$ at room temperature; the tunneling current was 1.24 nA.

The Au clusters on carbon-supported Pt nanoparticles were generated using the same method. Because the size of the Pt nanoparticles is about 3 nm, the Au clusters on Au/Pt/C are clearly much smaller than those on Au/Pt(111). We used the adsorption and oxidative desorption of CO to determine the Pt surface area blocked by Au. A thin layer of catalyst was bonded by a thin Nafion film to a glassy carbon disk of a rotating disk electrode. The CO stripping measurements on Pt/C and Au/Pt/C (fig. S1) revealed that the Au clusters in Au/Pt/C covered about 30 to 40% of the Pt surface. We assumed in this calculation that CO is not adsorbed on the Au surface under these conditions.

The structure of the Au-modified Pt/C catalyst was examined by high-resolution transmission electron microscopy. Measurements were performed using a high-resolution 300-kV field-

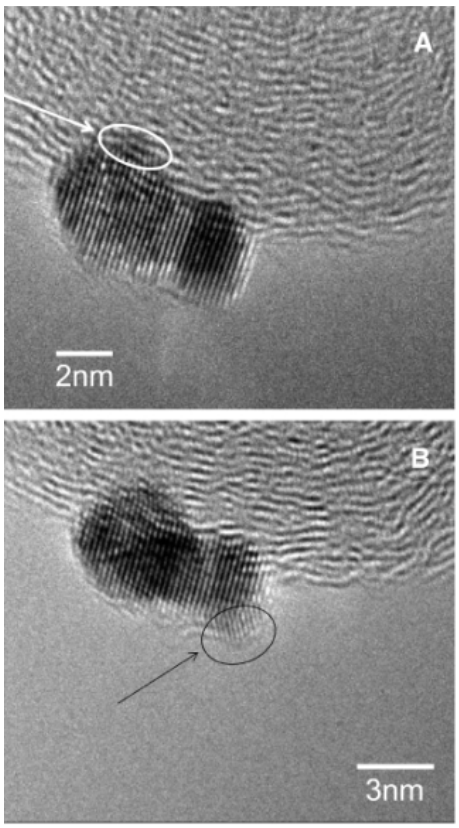

Fig. 2. Electron micrographs of a Au/Pt/C catalyst made by displacement of a Cu monolayer by Au. High-resolution images (**A** and **B**) show atomic rows with spacings that are consistent with the Pt(111) single-crystal structure. A different structure in the areas indicated by the arrows is ascribed to the Au clusters.

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emission microscope (JEOL3000F) equipped with an energy filter, an energy-dispersive x-ray spectrometer, and an electron energy-loss spectrometer. Low-magnification images (fig. S2) indicate the presence of metal particles averaging 3 to 5 nm in size on ~50-nm carbon spheres. Figure 2 shows the morphology of two isolated metal nanoparticles on the carbon support. Energy-dispersive spectroscopy applied directly to these particles showed the presence of 10 to 11% Au on Pt. The most frequently observed lattice fringes fit well with the Pt(111) surface. The distinct structures indicated by arrows in Fig. 2 are ascribed to the Au clusters, which appear amorphous rather than crystalline.

The in situ extended x-ray absorption fine structure (EXAFS) spectra (fig. S3) of the Pt L_3 and Au L_3 edges of the Au/Pt/C electrocatalyst have an absorption intensity at the Au L_3 edge (11,919 eV) that is ~28% of that at the Pt L_3 edge (11,564 eV). The difference between the absorption intensities approximates the composition of the Au/Pt electrocatalyst because of the proximity of Pt and Au absorption coefficients. The mole ratio of bulk atoms to surface atoms for the 2- to 3-nm size of these nanoparticles is ~40 to 50%. If a 2/3 monolayer of Au is deposited on the surfaces of Pt nanoparticles, the Pt:Au mole ratio must thus range from 1:0.26 to 1:0.33, which is in a very good agreement with the above result of 28% (that is, 1:0.28) from EXAFS spectra. Because Au L_3 absorption begins only 355 eV after the onset of the Pt L_3 edge, some fluctuations due to photoelectron scattering in the Pt EXAFS spectrum must be superimposed on the Au spectrum. The proximity of the Pt and Au L_3 edges makes the analysis of such spectra questionable. Thus, the size of the Au clusters could not be determined.

We found that the Pt nanoparticles retain their ORR activity crucial for fuel-cell catalysts after the deposition of Au clusters. On a rotating disc-ring electrode, the activities of Au/Pt/C and Pt/C differed by only 3 mV, expressed as the half-wave potential of these two surfaces (fig. S4). The small difference in the ring currents of the two surfaces corroborates this conclusion. In addition, the ring currents show a negligible generation of H_2O_2, indicating a four-electron reduction of O_2 to H_2O on both surfaces.

The stabilizing effect of Au clusters on Pt was determined in an accelerated stability test by continuously applying linear potential sweeps from 0.6 to 1.1 V, which caused surface oxidation/reduction cycles of Pt. The surface reaction involves the formation of PtOH and PtO derived from the oxidation of water that causes the dissolution of Pt via the Pt^{2+} oxidation state (*12*). We conducted the test by applying potential sweeps at the rate of 50 mV/s to a thin-layer rotating disk electrode in an O_2-saturated 0.1 M $HClO_4$ solution at room temperature. For comparison, a Pt/C catalyst with the same Pt loading as that in Au/Pt/C was subjected to the same potential cycling conditions. After 30,000 cycles, changes in the Pt surface area and electrocatalytic activity of the ORR were determined.

The catalytic activity of Au/Pt/C, measured as the currents of O_2 reduction obtained before and after potential cycling, showed only a 5-mV degradation in half-wave potential over the cycling period (Fig. 3A); in contrast, the corresponding change for Pt/C amounts to a loss of 39 mV (Fig. 3C). The same experiment with Au/Pt/C at 60°C showed no loss of activity (fig. S5), affording additional evidence for the stabilizing effect of Au clusters on the underlying Pt.

Voltammetry was used to determine the Pt surface area of the Au/Pt/C and Pt/C electrodes by measuring H adsorption before and after potential cycling. Integrating the charge between 0 and 0.36 V associated with H adsorption for Au/Pt/C shows no change, indicating no recordable loss of Pt surface area (Fig. 3B). However, for Pt/C, only ~55% of the original Pt surface area remained after potential cycling (Fig. 3D). As expected, the surface-area measurements are in good agreement with the measured ORR activities.

For the Pt/C catalyst (11.9 $\mu g_{Pt}/cm^2$), the measured degradation of the half-wave potential ($E_{1/2}$) after 30,000 cycles (at room temperature) was 39 mV. If the Pt specific activity does not vary significantly with the potential cycling, and assuming a constant Tafel slope b of –120 mV, the remaining Pt surface area after potential cycling can be estimated by using the expression (see the supporting online material) $\Delta E_{1/2} = -b \times \log(S_{Pt}/S_{Pt0})$, where S_{Pt} is the Pt surface area after cycling and S_{Pt0} is the initial Pt surface area. For the loss in $E_{1/2}$ of 39 mV, the calculated value for the remaining active Pt surface area is 47% of the initial one. This is less than the observed 55%, but the difference is not surprising given a possible change of the interfacial conditions during

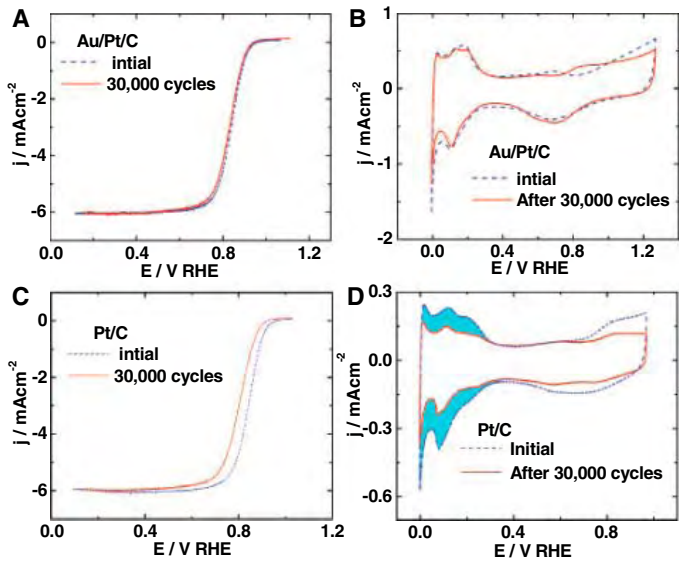

Fig. 3. Polarization curves for the O_2 reduction reaction on Au/Pt/C (**A**) and Pt/C (**C**) catalysts on a rotating disk electrode, before and after 30,000 potential cycles. Sweep rate, 10 mV/s; rotation rate, 1600 rpm. Voltammetry curves for Au/Pt/C (**B**) and Pt/C (**D**) catalysts before and after 30,000 cycles; sweep rate, 50 and 20 mV/s, respectively. The potential cycles were from 0.6 to 1.1 V in an O_2-saturated 0.1 M $HClO_4$ solution at room temperature. For all electrodes, the Pt loading was 1.95 μg (or 10 nmol) of Pt on a 0.164 cm^2 glassy carbon rotating-disk electrode. The shaded area in (D) indicates the lost Pt area.

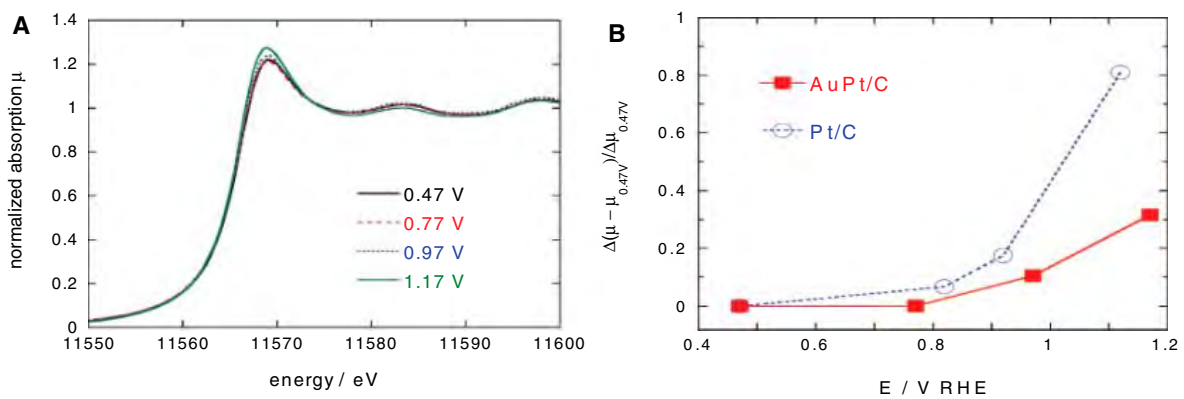

Fig. 4. (**A**) XANES spectra obtained with the Au/Pt/C catalyst at the Pt L_3 edge at four different potentials. (**B**) A comparison of the change of the absorption edge peaks of the XANES spectra for Au/Pt/C and Pt/C as a function of potential, obtained with the electrocatalysts at four different potentials in 1 M $HClO_4$.

30,000 cycles and the approximations involved in the calculation. A similar expression for the cell can be found in reference (*11*).

This stabilizing effect of Au clusters and lack of ORR inhibition, despite blockage of approximately one-third of the Pt sites on Au/Pt/C by Au, are intriguing phenomena that may have additional applications beyond fuel cells. To elucidate the origin of the observed stabilization effect of Au clusters, we determined by in situ x-ray absorption near edge spectroscopy (XANES) the oxidation state of Pt as a function of potential for the Au/Pt/C and Pt/C surfaces. The data offer strong evidence of decreased oxidation of Pt nanoparticles covered by Au in comparison with the oxidation of Pt nanoparticles lacking such coverage. In the XANES spectra for Au/Pt/C at the Pt L_3 edge (Fig. 4A), the intensity of the absorption bands reflects the depletion of the *d* band caused by the oxidation of Pt; a very small potential dependence indicates such oxidation. This effect is more evident in the relative change of the x-ray absorption peak intensity of the Pt L_3 edge spectra for Au/Pt/C and Pt/C as a function of potential (Fig. 4B). The increase in the intensity of the absorption edge peak for the Au/Pt/C electrocatalyst commences at considerably higher potentials than does that for the Pt/C catalyst; thus, the oxidation of Pt nanoparticles modified by Au clusters requires much higher potentials than are necessary for unmodified Pt nanoparticles. The high Pt oxidation potential of the Au/Pt/C electrocatalyst (that is, the lower extent of Pt oxidation) is clearly the major mechanism for the stabilization effect of Au clusters. A decreased Pt oxidation can also be discerned from a comparison of voltammetry curves for Au/Pt/C and Pt/C, as well as for Au/Pt(111) and Pt(111). The lower charge associated with the Pt oxidation at the potential region between 0.7 and 1.1 V clearly reveals the reduced oxidation of Au-modified surfaces (fig. S6). Table 1 gives a summary of the observed changes in surface area and catalytic activity data caused by potential cycling.

Nørskov and co-workers recently proposed a model describing the activity of metal adlayers (*14*, *15*), according to which the characteristics of the surface metal *d* bands, particularly the weighted center (ε_d), play a decisive role in determining surface reactivity. Density functional theory (DFT) calculations showed that the binding energies and reactivity of small adsorbates correlate well with the position of ε_d on strained surfaces and metal overlayers (*16*), in accordance with data from numerous experimental studies (*17*–*20*). The small Au clusters have more low-coordinate Au atoms than do the extended Au crystal surfaces. Au atoms with low coordination numbers have higher-lying *d* states, which are more reactive and interact more strongly with the adsorbate states (*21*). Au clusters on oxide supports can thereby activate molecular oxygen at room temperature (*22*–*24*).

When Au clusters are bound on a metallic, rather than oxide, substrate, the electronic interactions differ. Roudgar and Groβ (*25*) used DFT calculations to demonstrate a significant coupling of *d* orbitals of small Pd clusters to the Au(111) substrate. An equivalent type of interaction between Au and Pt can account for the observed stabilization of Pt. When clusters of the softer Au metal are placed on the surface of considerably harder Pt, there is practically no mixing between them. Del Pópolo *et al*. (*26*) previously reached a similar conclusion regarding Pd on an Au system. The surface alloying of Au with Pt, although unlikely, also would modify the Pt electronic structure toward a lower Pt surface energy, or lower-lying Pt *d*-band states.

The high ORR activity of the Au clusters on a modified Pt/C electrocatalyst is a counterintuitive observation. Au is not an active catalyst for the ORR to H_2O; instead, H_2O_2 is quantitatively generated in a two-electron reduction at most surfaces [except for Au(100) and its vicinal surfaces in alkaline solutions (*27*)]. Because Pt reduces O_2 to H_2O in a four-electron process, a decrease of the reduction current for the Pt surface that is one-third covered by a monolayer of Au would be expected. Without this decrease, it appears that Au clusters have a very high activity, in stark contrast to the behavior of the bulk Au or of carbon-supported Au nanoparticles. As discussed above, the mechanism of oxygen activation by Au clusters is controversial. Several researchers ascribed the activity of Au clusters to their interactions with oxides, resulting in charged Au particles (*28*). In view of our preceding discussion (*25*), such a process is not likely to occur with Au clusters at metal supports. To explain the observed activity, we might consider an efficient spillover of H_2O_2 from Au clusters to the surrounding Pt atoms, where further reduction to H_2O can take place. Alternatively, if AuOH is formed at certain potentials, it may help in reducing H_2O_2, as discussed for alkaline solutions (*29*). Such behavior could account for the negligible loss of activity of the Pt surface toward ORR.

Our studies raise promising possibilities for synthesizing improved ORR Pt-based catalysts and for stabilizing Pt and other Pt-group metals under oxidizing conditions.

Table 1. A comparison of surface area and the catalytic activity data for Pt/C and Au/Pt/C before and after 30,000 potential cycles from 0.6 to 1.1 V under the oxidizing conditions of the O_2 reduction reaction. Data were obtained from Fig. 3.

Catalyst and kinetic data	Pt dispersion (m^2/g_{Pt})	Half-wave potential at 1600 rpm (V)	Kinetic current density at 0.85 V (mA/cm^2)	Specific kinetic current density at 0.85 V (A/m^2_{Pt})
Pt/C initial	65.5	0.841	4.56	5.80
Pt/C after 30,000 cycles	35.5	0.802	1.60	3.72
Au/Pt/C initial	63.1	0.838	4.23	5.64
Au/Pt/C after 30,000 cycles	60.6	0.833	4.10	5.69

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Early Upper Paleolithic in Eastern Europe and Implications for the Dispersal of Modern Humans

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Radiocarbon and optically stimulated luminescence dating and magnetic stratigraphy indicate Upper Paleolithic occupation—probably representing modern humans—at archaeological sites on the Don River in Russia 45,000 to 42,000 years ago. The oldest levels at Kostenki underlie a volcanic ash horizon identified as the Campanian Ignimbrite Y5 tephra that is dated elsewhere to about 40,000 years ago. The occupation layers contain bone and ivory artifacts, including possible figurative art, and fossil shells imported more than 500 kilometers. Thus, modern humans appeared on the central plain of Eastern Europe as early as anywhere else in northern Eurasia.

Modern humans and their Upper Paleolithic industry (Aurignacian) spread rapidly across western and central Europe roughly 42,000 to 40,000 years ago; there is evidence for a slightly earlier influx in south central Europe (*1*). The early Aurignacian sites appear to represent the dispersal of modern humans from Africa into Europe [although their skeletal remains more than 30,000 years old are scarce in northern Eurasia (*2*)]. The initial spread of the Upper Paleolithic is difficult to date because this event lies near the limit of effective ^{14}C dating and a major radiocarbon plateau (*3*). A volcanic ash deposited about 40,000 years ago (the CI tephra) provides a stratigraphic marker in parts of southern and eastern Europe (*4*). On the Don River in Russia, Aurignacian artifacts are buried within and beneath the CI tephra at Kostenki (*5*, *6*). Below the tephra lie traces of a local Upper Paleolithic industry that appears to date as early as 45,000 to 42,000 years ago (*7*) and contains typical Upper Paleolithic tool forms, personal ornaments, carved ivory (possible figurative art), and raw materials imported from distant sources (*8*, *9*). The artifacts probably were made by modern humans, although skeletal remains are confined to isolated teeth. The Kostenki discovery indicates the presence of a fully developed Upper Paleolithic industry on the central East European Plain as early as anywhere in northern Eurasia, and it has implications for both the timing and routes of modern human dispersal.

Kostenki is located ~400 km south of Moscow on the west bank of the Don River, which is deeply incised by ravines (Fig. 1). Most of the Paleolithic sites are found on low terraces near the mouths or the upper reaches of these ravines (*10*, *11*), all of which contain active springs. A total of 21 sites—most of them comprising several occupation levels—are known at Kostenki. An additional seven sites now are recorded near Borshchevo, several kilometers to the southeast (*11*–*13*) (Fig. 2).

The west bank of the Don Valley is composed of Cretaceous marl and sand that unconformably overlie Devonian clay (*14*–*16*). Although several Upper Paleolithic sites (e.g., Kostenki 18) are located on the third terrace (30 to 40 m) in late Pleistocene loams that directly overlie Cretaceous sand, most sites are found on the second (15 to 20 m) or first (10 m) terrace (*10*–*12*). Sites that contain Upper Paleolithic artifacts dating to the interstadial that correlates with Marine Isotope Stage 3 (60,000 to 30,000 years ago) are confined to the second terrace. Alluvium of this terrace was deposited by the Don River earlier than 50,000 years ago and rests unconformably on the Devonian clay (*17*). Above the alluvium is a sequence of interbedded lenses of silt, carbonate, chalk fragments, and organic-rich loam ("humic beds") that date to ~50,000 to 30,000 years ago

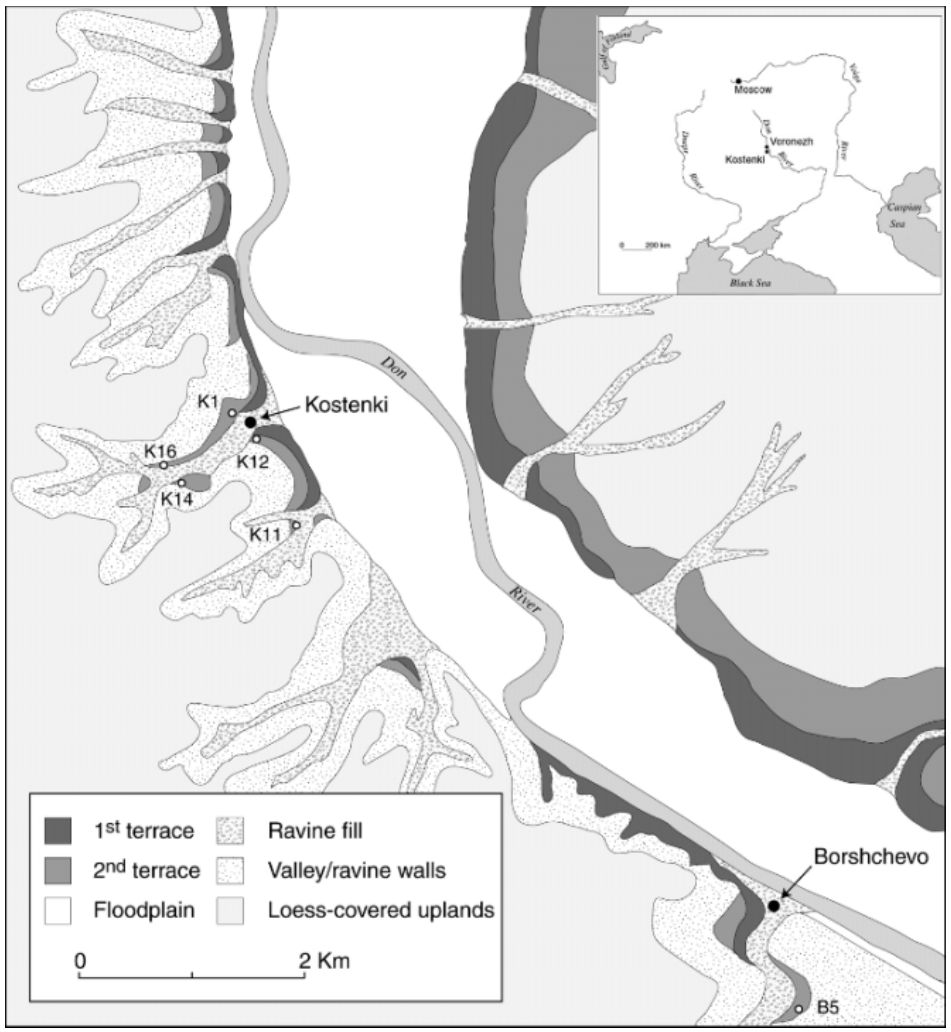

Fig. 1. Map of the Kostenki-Borshchevo area (*14*, *17*), showing the location of Upper Paleolithic sites investigated during 2001–2004. Inset: location of Kostenki.

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(*18*) (tables S1 and S2). Micromorphology analysis indicates that the carbonate is primary and probably derived from local springs and seeps, whereas the chalk fragments represent eroded Cretaceous marl washed and soliflucted from higher slopes (Fig. 3) (table S3).

The humic beds are subdivided by the Campanian Ignimbrite (CI) Y5 tephra (*5*, *19*). The CI tephra has an $^{40}Ar/^{39}Ar$ date of 41,000 to 38,500 years ago and is stratigraphically correlated with the onset of Heinrich Event 4, as well as with Laschamps geomagnetic excursion and a related cosmogenic nuclide peak (*4*, *20*). At Kostenki 12, sediment below the level of the ash horizon yielded optically stimulated luminescence (OSL) dates of between 52,440 ± 3850 and 45,200 ± 3260 years (table S4). Paleomagnetic measurements show that this sediment contains the Laschamps excursion, which has been dated elsewhere to 45,000 to 39,000 years ago (*21*, *22*). Calibration of conventional and AMS radiocarbon dates obtained on charcoal (*23*) with two long curves (*24*, *25*) yields a similar chronology, although calibrated dates above and below the CI tephra are roughly 2000 years younger than ages determined by other methods (table S5).

Fig. 2. Excavations at Kostenki 14 in 2002, showing the north and east walls.

Artifacts assigned to the Upper Paleolithic have been recovered from all levels of the humic beds (including the tephra horizon at Kostenki 14) and indicate substantive occupation of the Kostenki area before 40,000 years ago. Human skeletal remains found below the tephra are confined to two isolated teeth, which are tentatively assigned to modern humans (*Homo sapiens*) (*8*, *9*). More complete skeletal remains that can be firmly attributed to modern humans have been recovered from the humic layers (and a buried soil at Kostenki 1) above the CI tephra and dated to ≥30,000 years ago (*23*).

Although generally rare in eastern Europe, assemblages that may be assigned to the Aurignacian industry are present at Kostenki (*26*). An early Aurignacian assemblage containing end-scrapers, *lamelles Dufour*, and ornaments of shell and bone was recovered from the CI tephra at Kostenki 14 (i.e., ~40,000 years ago) (*6*). Younger Aurignacian artifacts are associated with the buried soil above the tephra at Kostenki 1 and date to ~30,000 years ago (*7*, *12*).

Occupation layers buried below the CI tephra at Kostenki 1, 12, 14, and 17 contain Upper Paleolithic assemblages that precede the local Aurignacian. Pollen stratigraphy at Kostenki 12 (which exhibits good agreement with the magnetic characteristics of the sediment) indicates that the interbedded silts and organic loams containing these assemblages accumulated during a series of warm and cold oscillations during MIS 3 before the CI eruption (*27*, *28*). The period of maximum warmth corresponds to the lowest artifact-bearing units and—on the basis of stratigraphic position and OSL dates—is correlated with GIS 12 in the Greenland ice record (with an estimated age of 45,000 years in GISP2) (*29*), which appears to represent a lower limiting date for Upper Paleolithic occupation at Kostenki.

At least two types of assemblages are found below the CI tephra. At Kostenki 14, the lowermost occupation level (Layer IVb) contains prismatic blade cores, bladelets, end-scrapers, burins, *pièces ésquillées*, and small bifaces. Nonstone artifacts include bone points, antler mattocks, worked ivory, and perforated shell ornaments (Fig. 4). One carved piece of ivory appears to represent the head of an (unfinished) human figurine (Fig. 4E) (*8*). At Kostenki 17, Layer II yielded large prismatic blades, numerous burins, end-scrapers, and some *pièces ésquillées*. Ornaments of stone were perforated with a hand-operated rotary drill (*9*). Nonstone items include bone points and awls and some worked ivory. These assemblages are associated with large numbers of small and medium mammal remains—especially hare (*Lepus tanaiticus*), arctic fox (*Alopex lagopus*), and wolf (*Canis lupus*)—and some bird remains. The bones and teeth of large mammals, including horse (*Equus latipes*) and

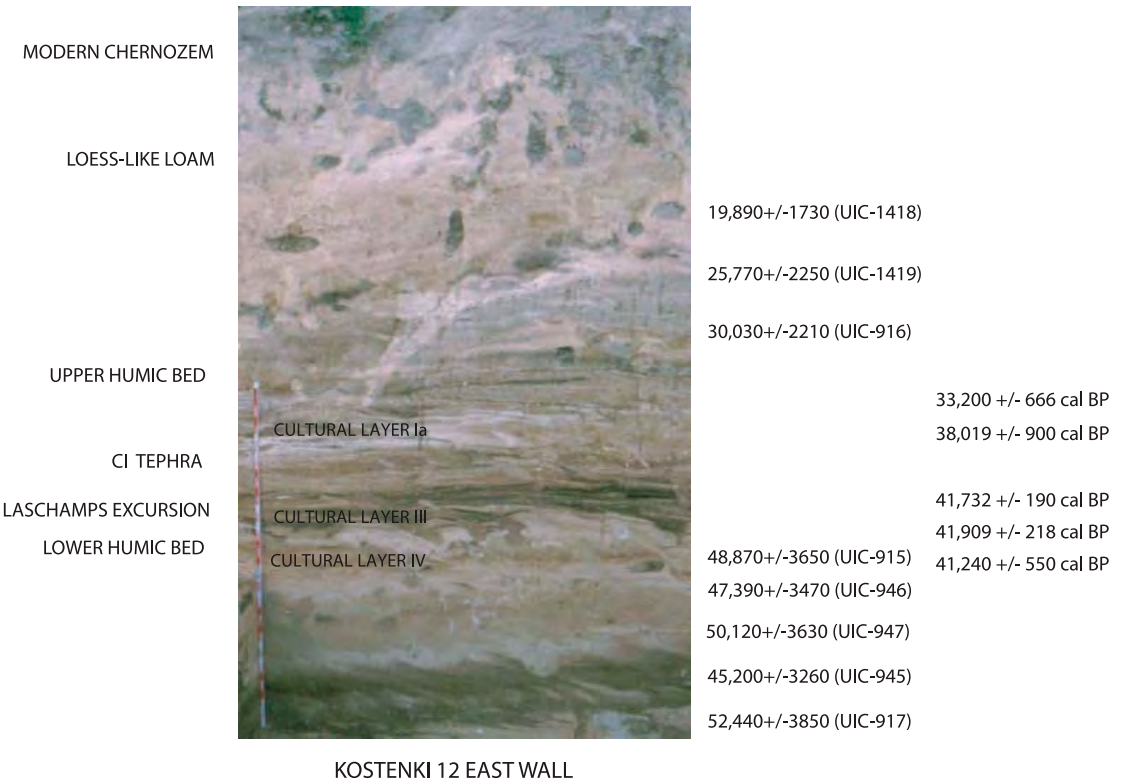

Fig. 3. Stratigraphic profile of Kostenki 12 (east wall) showing the position of the humic beds, CI tephra horizon, Laschamps excursion, and Upper Paleolithic cultural layers, as well as OSL dates and calibrated radiocarbon dates on charcoal.

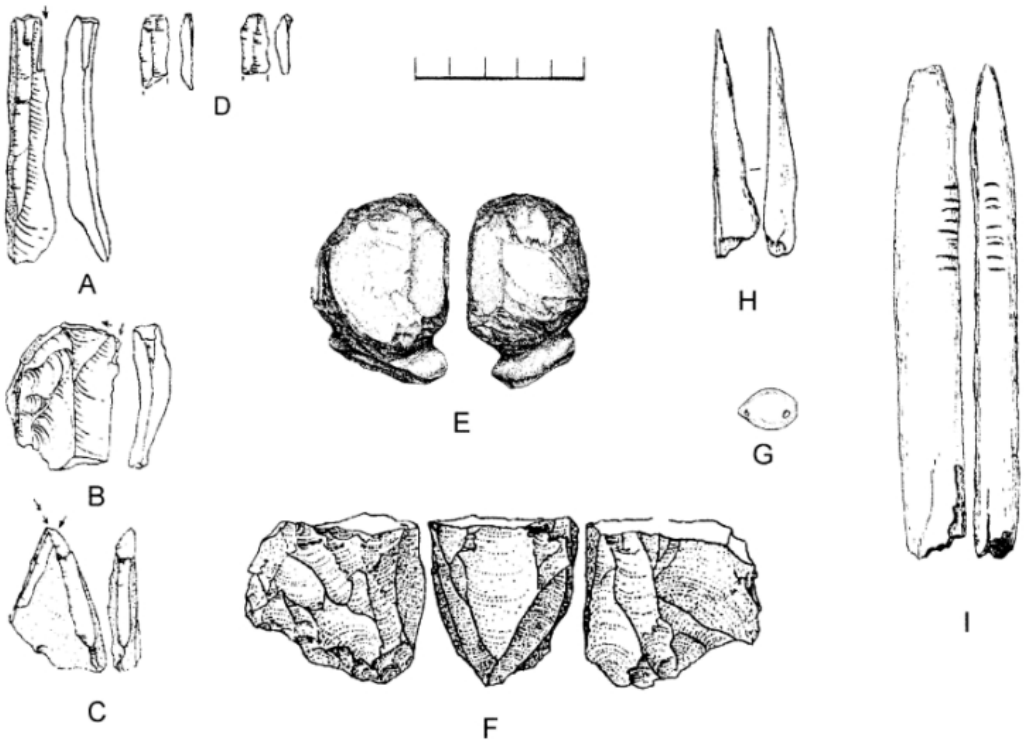

Fig. 4. Stone, ivory, and bone artifacts from the lowest Upper Paleolithic horizon at Kostenki (Kostenki 14, Layer IVb): (**A** to **C**) burins; (**D**) small bade fragments; (**E**) carved ivory fragment possibly representing the head of an unfinished human figurine; (**F**) blade core; (**G**) perforated fossil shell; (**H**) bone awl; (**I**) bone point. Scale bar = 5 cm.

reindeer (*Rangifer tarandus*), are present but less common (*30*).

The artifact assemblages below the CI tephra do not represent an Upper Paleolithic industry that is "transitional" from the local Middle Paleolithic, but rather an abrupt departure from the latter. Prismatic blade technology is predominant and Middle Paleolithic artifact types are rare. Most of the stone used for artifact production was imported 100 to 150 km from its sources (*9*), and the perforated shells (Columbellidae) in the lowermost level at Kostenki 14 (Fig. 4G) apparently are derived from a source no closer than the Black Sea (i.e., transported >500 km) (*8*). Other raw materials include bone, antler, and ivory. Most noteworthy is the carved ivory piece that may represent an example of figurative art. Novel technologies include the rotary drill and—by implication—devices for harvesting small game (*26*). Although taxonomic assignment of the associated human teeth is tentative, the contents of this Upper Paleolithic industry suggest that it was probably manufactured by modern humans.

Deposits below the CI tephra at Kostenki also yielded several artifact assemblages that primarily contain typical Middle Paleolithic tool forms (e.g., side-scrapers, bifaces) manufactured on flakes (*7*). They lack imported raw materials, bone-antler-ivory artifacts, and art. The faunal remains are confined to large mammals (*30*). These assemblages, which are assigned to the local Strelets culture, are analogous to the "transitional" Upper Paleolithic industries of western and central Europe (especially the Szeletian), at least some of which apparently were produced by local Neandertals (*1*, *26*). The Strelets artifacts are not associated with any human skeletal remains and their makers are unknown. They may represent an activity variant of the other Kostenki industry (i.e., probably produced by modern humans) related to the butchering of large mammals. Younger Strelets assemblages are found above the CI tephra (*7*, *12*).

The developed (i.e., nontransitional) Upper Paleolithic industry in the lowest occupation levels of Kostenki 14 and 17 appears to represent an intrusion of modern humans onto the central East European Plain several thousand years before their spread across western and eastern Europe. It is not clear whether Neandertals also occupied the central East European Plain at this time (although they were present in other parts of eastern Europe) (*26*), and both climate and the presence of human competitors might have played a role in the early appearance of modern humans on the middle Don River. Also unclear is the relationship between the Kostenki industry and the earliest dated Upper Paleolithic remains in south central Europe, which appear to be of comparable age (*1*, *4*). Although broadly similar, the early Upper Paleolithic assemblages of each region may represent separate routes of dispersal for modern humans entering Europe.

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Tables S1 to S5
References

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Late Pleistocene Human Skull from Hofmeyr, South Africa, and Modern Human Origins

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The lack of Late Pleistocene human fossils from sub-Saharan Africa has limited paleontological testing of competing models of recent human evolution. We have dated a skull from Hofmeyr, South Africa, to 36.2 ± 3.3 thousand years ago through a combination of optically stimulated luminescence and uranium-series dating methods. The skull is morphologically modern overall but displays some archaic features. Its strongest morphometric affinities are with Upper Paleolithic (UP) Eurasians rather than recent, geographically proximate people. The Hofmeyr cranium is consistent with the hypothesis that UP Eurasians descended from a population that emigrated from sub-Saharan Africa in the Late Pleistocene.

Most genetic studies indicate that all contemporary humans owe their ancestry to a sub-Saharan African population, extant between 100 and 200 thousand years ago (ka) (*1*–*3*). A number of genetic studies further suggest that modern humans left sub-Saharan Africa in the Late Pleistocene, between 65 and 25 ka (*1*–*6*). The middle of this range (~45 to 35 ka) corresponds not only with the appearance of Later Stone Age (LSA) industries in sub-Saharan Africa (*7*) but also with the earliest Upper Paleolithic (UP) industries and human skeletons in Eurasia (*8*). However, other genetic data appear to suggest substantial non-African contributions to the genomes of modern human populations, and these data have been interpreted as being inconsistent with any population bottleneck associated with a recent African exodus (*9*, *10*).

The human palaeontological record might be used to test predictions from these hypotheses. Craniometric data tend to differentiate recent human populations in accord with their geographic distributions and genetic relationships (*11*–*15*). Eurasian UP crania do not particularly resemble those of earlier Eurasian Neandertals (*16*), nor are they especially similar to recent human crania from sub-Saharan Africa (*12*). Thus, we should not expect to see any special similarity between the UP Eurasians and contemporaneous sub-Saharan Africans in the absence of a Late Pleistocene exodus from sub-Saharan Africa.

Although there are several variably complete crania from North Africa that date to between about 40 and 20 ka (from Dar es Soltan, Morocco; and Nazlet Khater and Wadi Kubbaniya, Egypt), the only sub-Saharan specimen in LSA context that has been claimed to pre-date 20 ka is an infant mandible from Origstad Shelter, South Africa, and it may be substantially younger (*17*). The lack of Late Pleistocene human remains from sub-Saharan Africa has resulted in an inability to test competing models of human evolution (*18*).

We report on a nearly complete human cranium from Hofmeyr, South Africa, and its dating to 36.2 ± 3.3 ka. The skull was discovered in 1952 in a dry channel bed of the Vlekpoort River (25°58'E, 31°34'S) near the town of Hofmeyr, Eastern Cape Province, South Africa. The endocranial cavity, orbits, nasal cavity, and palate were filled with an indurated carbonate-sand matrix. No other bones or archaeological artefacts were reportedly found in the vicinity at the time of the skull's discovery, and within a decade, the channel had become filled by silt, after the construction of an anti-erosion weir downstream. This precludes any possibility of locating the original position of the specimen or of directly dating the surrounding sediments.

In the 1960s, a substantial portion of the left parietal bone was removed, presumably in an attempt to obtain a radiocarbon date, although no date has ever been published. Another, smaller bone sample was submitted by us to the University of Oxford Radiocarbon Accelerator Unit to assess its amenability to accelerator mass spectrometry (AMS) ^{14}C dating, but it lacked sufficient collagen for an accurate age determination (*19*). Instead, we estimated the burial age of the skull by dating the residence time of the matrix filling the endocranial cavity, using a combination of optically stimulated luminescence and uranium-series dating methods, coupled through a radiation-field model. The length of time between death and incorporation of the sediment within the skull is expected to be short, because the loss of organic material after death would be rapid (days to months). Furthermore, the skull's relatively good state of preservation suggests that it had neither been uncovered long before nor transported any substantial distance before its discovery (the force required to scour the innermost sediments would certainly have resulted in substantial damage). Additional evidence for a single infilling episode comes from the consistency of the dates determined from the samples of endocranial matrix.

The signals measured in luminescence dating are a consequence of the absorption by mineral grains of ionizing radiation from low concentrations of radionuclides that are naturally present in the sediment and from cosmic rays (*20*). Luminescence dating methods provide estimates of the total ionizing radiation dose [D_e, in units of grays (Gy)] absorbed by sediment (in this case quartz) grains since their burial. Estimation of burial age is possible if the radiation dose rate (D', in units of Gy/ka) is known. In the simplest case, where D' is constant in time, age = D_e/D'. Three samples of endocranial sediment were extracted

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under red-light conditions from the central portion of the endocranial cavity, and successful measurements of D_e were made on aliquots of refined quartz from each sample. Estimation of D' for the sampled grains is more complex than is typically the case in luminescence dating, due mainly to the presence of carbonate in the sediment and to a measured disequilibrium in the U decay series. Carbonate has the potential to reduce D' by radiation attenuation or to increase it by the incorporation of mobile U. A further complication is that bone adsorbs mobile U; thus, the skull itself may contribute an additional local γ-dose component to D'. These factors combine to give both a time and space dependence to D'. The date of carbonate formation was assessed as 24.0 ± 5.2 ka with the use of a Th/U isochron (*21*), and the deposition of energy within the interior of the skull (giving D') during burial was modeled with a three-dimensional (3D) Monte Carlo radiation transport model (*22*, *23*) (with the model geometry defined with data from a computed tomography scan of the skull). From this modeling, the time required for the total radiation dose (defined by the luminescence measurements) to have been absorbed by the quartz grains could be estimated, yielding ages for the three samples of 40.9 ± 4.2, 33.0 ± 2.5, and 34.7 ± 3.4 ka. These combine as 36.2 ± 3.3 ka (1σ) for the depositional age of the endocranial sediment (*24*).

The skull has suffered post-recovery mishandling, with the resultant loss of the anterior part of the lower facial skeleton, the angle of the mandible, the mastoid process of the right temporal, and much of the occipital. However, it was photographed and measured before this damage (Fig. 1), and there is no distortion of the remaining parts of the cranium. We incorporated the missing parts from these photographs and used measurements recorded before the damage.

The Hofmeyr skull is fully adult; the coronal suture is obliterated and the third molars are heavily worn. It suffered antemortem trauma to the lateral margin of the right orbit, which exhibits a healed or partially healed depressed fracture. This crushing, together with associated bony exostoses along its posterior margin, exaggerate the thickness of the frontal process of the zygomatic. The anterior surface of the right supraorbital torus appears to have been cut away.

Hofmeyr presents an overall picture of morphological modernity in its steeply rising frontal and high rounded vault, the maximum breadth of which is situated high on the parietals. Weak frontal eminences recede laterally from a broad low midline keel that rises vertically from the glabella. The skull is large and robust. The maximum estimated length and breadth of the neurocranium, as well as most measurements of the facial skeleton, lie at or exceed two standard deviations (SD) of the means for modern African males, whereas they lie within these limits for Late Pleistocene crania from Eurasia and North Africa (table S3). Narrow nasal bones are bounded by very broad (~15.0 mm), relatively flat frontal processes of the maxillae. The pyriform aperture is broad in comparison to that of most Eurasian UP crania. The infraorbital plate is tall and flat and lacks an inframalar curve. As such, it differs from the condition that characterizes recent southern African crania (*12*, *25*). Frontal and parietal thickness (6 to 7 mm) is comparable to that of recent humans.

The glabella projects to a greater degree than in modern Africans but is comparable to that of UP crania. The supraorbital tori of Hofmeyr are moderately well developed and continuous, lacking the separation of the medial supraorbital eminence and lateral superciliary arch that is characteristic of recent humans. Although the supraorbital torus is comparable in thickness to that in UP crania, its continuous nature represents a more archaic morphology (*26*). In this regard, Hofmeyr is more primitive than later sub-Saharan LSA and North African UP specimens (such as Lukenya Hill and Wadi Kubbaniya), even though they may have a somewhat thicker medial supraorbital eminence. Despite its glabellar prominence and capacious maxillary sinuses, Hofmeyr exhibits only incipient frontal sinus development, a condition that is uncommon among European UP crania (*27*).

The mandibular ramus has a well-developed gonial angle, and the slender coronoid process is equivalent in height to the condyle. The mandibular (sigmoid) notch is deep and symmetrical, and its crest intersects the lateral third of the condyle. The anterior margin of the ramus is damaged, but it is clear that there was no retromolar gap.

The Hofmeyr molars are large. The buccolingual diameter of M^2 exceeds recent African and Eurasian UP sample means by more than 2 SD (table S3). Radiographs reveal cynodont molars,

Fig. 1. The Hofmeyr skull in facial (top row), right lateral (middle row), and superior (bottom row) views as it appeared in 1968 and 1998. Note the initial state of preservation of the skull, and the antemortem damage to the supraorbital torus and lateral margin of the right orbit.

although pulp chamber height is likely to have been affected by the deposition of secondary dentine in these heavily worn teeth.

Thus, Hofmeyr is seemingly primitive in comparison to recent African crania in a number of features, including a prominent glabella; moderately thick, continuous supraorbital tori; a tall, flat, and straight malar; a broad frontal process of the maxilla; and comparatively large molar crowns. Hofmeyr is contemporaneous with later Eurasian Neandertals, but it clearly does not evince the cranial and mandibular apomorphies that define that clade (*28*). This is not surprising, given its geographic location. Although Hofmeyr is similar in size to Eurasian UP crania, it differs from them in other respects (such as its broad nose and continuous supraorbital tori).

In order to assess the phenetic affinities of Hofmeyr to penecontemporaneous Eurasian UP and recent humans, we conducted multivariate morphometric analyses of 3D landmark coordinates and linear measurements of crania representing these populations. We digitized 19 3D coordinates of landmarks that represent as fully as possible the currently preserved anatomy of the Hofmeyr skull (table S4). These were compared with homologous data for recent human samples from five broad geographic areas (North Africa, sub-Saharan Africa, Western Eurasia, Oceania, and Eastern Asia/New World). The sub-Saharan sample was divided into Bantu-speaking (Mali and Kenya) and South African Khoe-San samples. The latter are represented in the Holocene archaeological record of the subcontinent, and inasmuch as they are the oldest historic indigenes of southern Africa, they might be expected to have the closest affinity to Hofmeyr (*12*). The North African sample consists of Epipaleolithic (Mesolithic) individuals that provide a temporal depth of approximately 10,000 years. The 3D data were also compared for two Neandertal, four Eurasian UP, and one Levantine early modern human fossils (table S5). The landmark coordinate configurations for each specimen were superimposed with the use of generalized Procrustes analysis and analyzed with a series of multivariate statistical techniques (*29*).

Hofmeyr falls at the upper ends of the recent sub-Saharan African sample ranges and within the upper parts of all other recent human sample ranges in terms of centroid size (fig. S6). In a canonical variates analysis of these landmarks (Fig. 2), axis 1 separates the sub-Saharan African samples from the others, and axis 4 tends to differentiate the UP specimens from recent homologs. Hofmeyr clusters with the UP sample, and although it falls within the recent human range on both axes, it is outside the 95% confidence ellipse for the Khoe-San sample and barely within the limits of the other sub-Saharan African sample. These canonical axes are weakly correlated with centroid size, which emphasizes that the similarity between Hofmeyr and the UP sample is due only in small part to similarity in size.

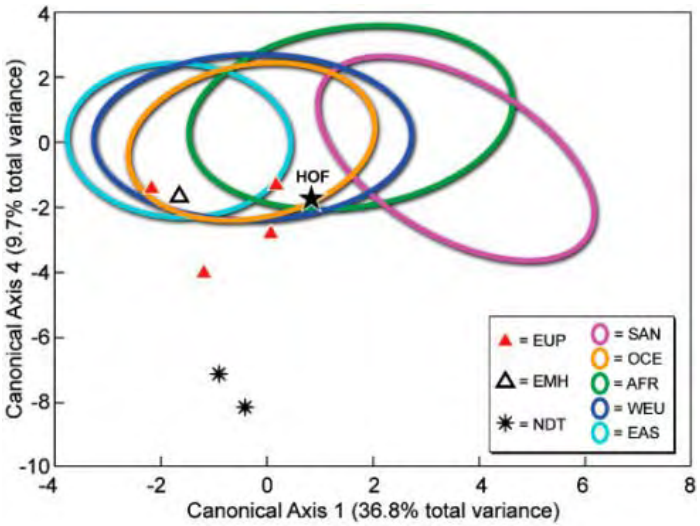

Fig. 2. Canonical variates analysis of recent and fossil samples showing 95% confidence ellipses for recent samples. Symbol abbreviations are as follows: HOF (Hofmeyr), EUP (Eurasian Upper Paleolithic), EMH (Skhūl 5 early modern human), and NDT (Neandertals). Ellipses represent 95% confidence ellipses for recent human samples: SAN (South African Khoe-San), AFR (sub-Saharan Africa), WEU (Western Eurasia), OCE (Oceania), and EAS (East Asia/New World).

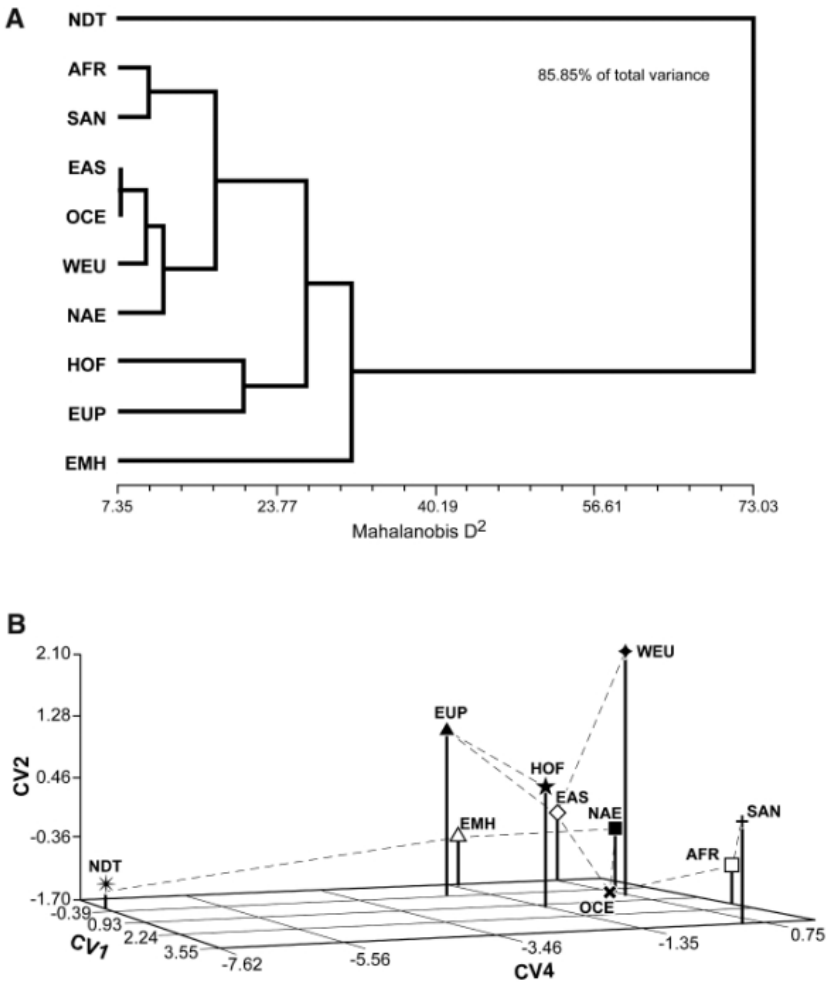

Fig. 3. Phenetic affinities of the Hofmeyr (HOF) cranium determined from 3D coordinates landmarks. (**A**) UPGMA tree based on Mahalanobis D^2 distances (corrected for unequal sample sizes) among samples. (**B**) Minimum spanning tree representing the closest links based on the total variance of all 21 principal components from which the canonical variates analysis was calculated. Sample abbreviations are as follows: AFR (sub-Saharan Africa), EAS (East Asia/New World), EMH (early modern human = Skhūl), EUP (Eurasian Upper Paleolithic), NAE (North African Epipaleolithic), NDT (Neandertal), OCE (Oceania), SAN (South African Khoe-San), and WEU (Western Eurasia).

Mahalanobis squared distances among samples were calculated to establish the group to which Hofmeyr has the greatest similarity (table S6). Hofmeyr shows low posterior and typicality probabilities for all recent humans, but much higher probabilities for the UP sample (posterior 0.76, typicality 0.43). The unweighted pair group method with arithmetic average (UPGMA) cluster and 3D minimum spanning trees calculated from the Mahalanobis generalized (D^2) distances highlight Hofmeyr's phenetic affinity to the UP specimens and its distinction from recent sub-Saharan Africans (Fig. 3). The high success rate of cross-validation classification lends confidence to these results: ~ 80% of recent human crania are correctly classified to their geographic sample when this approach is used (*29*).

We sought to further assess the relationship between the Hofmeyr cranium and samples of various recent sub-Saharan Africans ($n = 263$) and Europeans ($n = 24$) and a small sample of UP Eurasians ($n = 5$), using eight linear dimensions of the face and cranial vault (table S7). The recent sub-Saharan African samples consisted of several Bantu-speaking groups that were combined because no significant differentiation among them was observed through analyses of variance.

The craniofacial variables were size-adjusted by transforming them into Z- and C-scores following Howells (*11*) and were analyzed by factor analysis with varimax rotation following Ribot (*13*). Analyses of variance of the regression factor scores indicate that factor 2 provided the greatest differentiation among the comparative samples. Therefore, this was used preferentially to identify the position of Hofmeyr vis-à-vis the 95% confidence ellipses of these samples. Hofmeyr is encompassed by the variation exhibited by Late Pleistocene Eurasian crania (Fig. 4). It is also encompassed by the 95% confidence ellipse of the recent Khoe-San and sub-Saharan Bantu-speaker samples, but falls just beyond the 95% confidence ellipse of recent Europeans. These observations are supported by the proximity matrix of squared Euclidean distances derived from the regression factor scores, which reveal the UP Eurasian sample as closest to Hofmeyr (table S8).

Hofmeyr and the UP Eurasian specimens tend to have comparatively high loadings on factor 2, which is indicative of a trend toward relatively longer crania with relatively shorter orbits than those in recent populations from these same geographic areas. This perhaps attests to a common trend for change in craniofacial shape over the past 36,000 years in both Eurasia and sub-Saharan Africa.

The results of the 3D geometric and linear morphometric analyses suggest that Hofmeyr shares close affinity with Eurasian UP specimens but is more distant from recent sub-Saharan African populations. These analyses emphasize that neither large absolute size nor allometrically related shape similarities are responsible for the relationship seen between Hofmeyr and pene-contemporaneous Eurasian UP skulls.

The placement of Hofmeyr with Eurasian UP crania rather than with recent, geographically proximate humans is important given the specimen's geochronological age and the ability of craniometric data to differentiate recent human populations in accord with their geographic and genetic relationships. Our findings are consistent with the hypothesis that UP Eurasians descended from a population that emigrated from sub-Saharan Africa in the Late Pleistocene. The Hofmeyr cranium affords potential insights into the morphology of such a population.

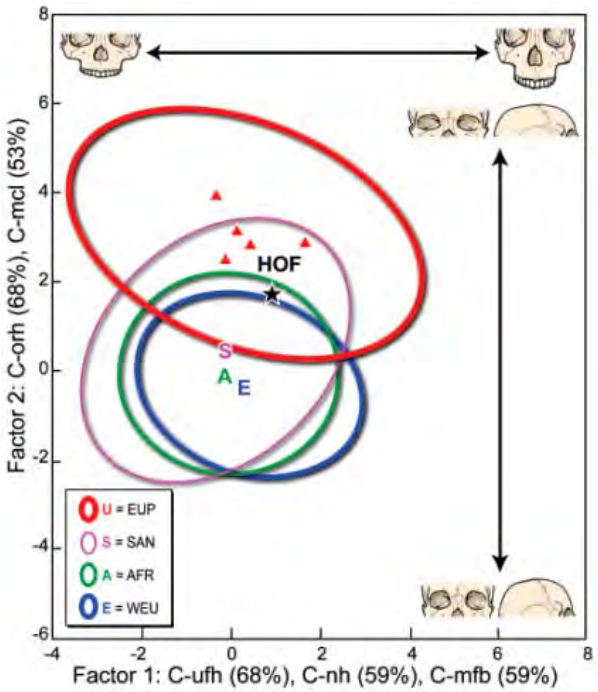

Fig. 4. Plot of regression factor scores of linear measurements recorded for Hofmeyr (HOF, star), Eurasian UP crania (EUP, triangles), and recent Khoe-San (SAN), sub-Saharan African (AFR), and Western Eurasian (WEU) samples. The 95% confidence ellipses for the UP and recent samples and the centroids of the recent samples (letters S, A, and E) are shown. This analysis expresses 50% of the total variance in the first two factors (factor 1 = 29%, factor 2 = 21%). Factor 1 accounts for 68% of the variance of upper facial height (C-ufh), 59% of the variance of nasal height (C-nh), and 59% of the variance of minimum frontal breadth (C-mfb). Factor 2 accounts for 68% of the variance of orbital height (C-orh) and 53% of the variance of maximum cranial length (C-mcl). Crania to the right of the plot exhibit tall faces, and crania to the bottom of the plot exhibit long vaults and short orbits. The samples are described in (*29*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5809/226/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S8
References

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Regulation of γδ Versus αβ T Lymphocyte Differentiation by the Transcription Factor SOX13

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αβ and γδ T cells originate from a common, multipotential precursor population in the thymus, but the molecular mechanisms regulating this lineage-fate decision are unknown. We have identified *Sox13* as a γδ-specific gene in the immune system. Using *Sox13* transgenic mice, we showed that this transcription factor promotes γδ T cell development while opposing αβ T cell differentiation. Conversely, mice deficient in *Sox13* expression exhibited impaired development of γδ T cells but not αβ T cells. One mechanism of SOX13 function is the inhibition of signaling by the developmentally important Wnt/T cell factor (TCF) pathway. Our data thus reveal a dominant pathway regulating the developmental fate of these two lineages of T lymphocytes.

T cells of the αβ and γδ lineages arise at different stages of ontogeny, preferentially localize to specific tissues, and perform distinct and overlapping immune functions (*1*, *2*). However, aside from their distinct cell-surface antigen-specific T cell receptors (TCRs), relatively little is known about the molecular characteristics that distinguish them. A central question in understanding how the two lineages of T cells arise is whether lineage choice in uncommitted progenitors is directed by distinct instructional signals from the γδTCR or the preTCR (a complex of TCRβ and the surrogate α chain) (*3*, *4*), or if precursors are pre-committed to either lineage before TCR expression (*5*, *6*). Because definitive markers to distinguish αβ and γδ lineages before TCR expression have been lacking, this question has been difficult to resolve.

To investigate the lineage-fate decision process at the molecular level, we screened for transcription factors (TFs) that were differentially expressed between αβ and γδ thymocytes and found that *Sox13*, a high-mobility group (HMG) TF (*7*), is a γδ T cell lineage–restricted molecule (Fig. 1A) (*8*). T cell progenitors that seed the thymus follow a defined, multistep differentiation program and are identified by the absence of cell-surface CD3, CD4, and CD8 [triple negative (TN)] molecules. TN cells are further subdivided into TN1 to TN4 by the cell-surface expression pattern of CD25 and CD44. Successful rearrangement and production of the TCRβ chain at the TN3 stage allows αβ-specific preTCR signaling, promoting differentiation into double-positive (DP, $CD4^+CD8^+$) immature αβ-lineage cells. γδ T cell differentiation diverges from this developmental progression between the TN2 and TN4 stages. In the TN population, *Sox13* expression was highest in the multipotent progenitors, TN1 and TN2 (Fig. 1B). Its expression was subsequently turned off in differentiating αβ-lineage cells starting from the TN3 stage (Fig. 1B). In the peripheral lymphoid organs, *Sox13* expression was detected in γδ T cells but not in αβ T, NK, or B cells (Fig. 1C). In contrast, the expression of other HMG TFs—*Tcf1*, *Lef1*, and *Sox4*—was detectable in both αβ- and γδ-lineage cells (Fig. 1D).

Sox13 was expressed in γδ cells of $Tcr\beta^{-/-}$ mice and in early fetal thymocytes well before the appearance of DP thymocytes (Fig. 1E) (*7*), thereby distinguishing it from other γδ-lineage–biased genes (such as *Rgs1*) whose expression is dependent on conventional DP thymocytes (*9*). *Sox13* expression is also not linked to the specific activation state of lymphocytes shared by "nonconventional" lymphocytes because it was not expressed in αβ intestinal intraepithelial lymphocytes (IELs) (Fig. 1C). Further, *Sox13* expression is not linked to TCR rearrangement or expression, indicating that *Sox13* expression in the immune system strictly correlates with T cell sublineage but not TCR type (fig. S1).

Expression of *Sox13* in TN2 cells is consistent with its potential role in early γδ T cell development. These precursors do not exhibit substantial levels of TCR gene rearrangement, and we have previously shown that the developmental potential of TN2 cells is heterogeneous (*10*). These data, in combination with

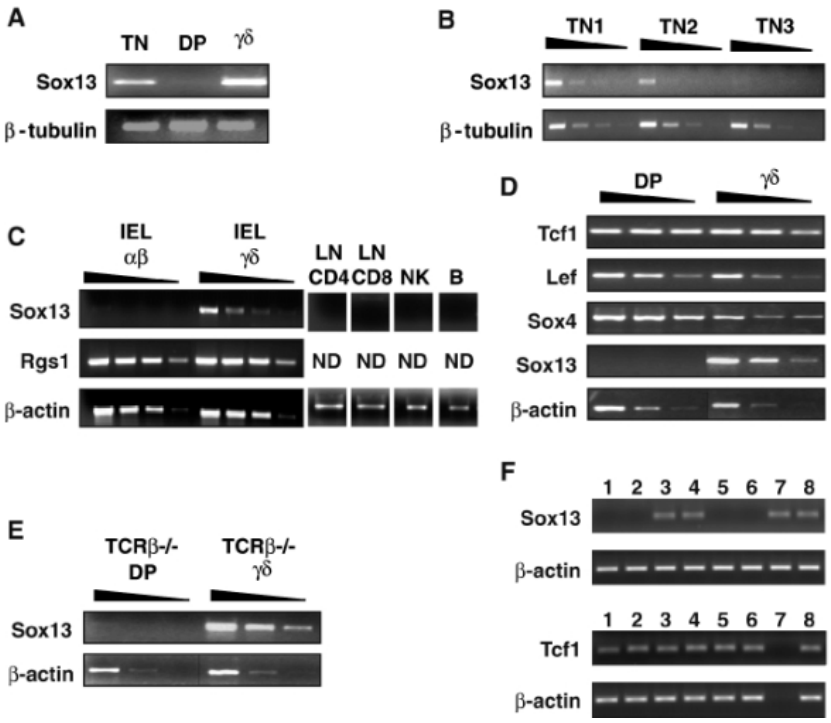

Fig. 1. *Sox13* expression in thymocytes. (**A**) Reverse transcription polymerase chain reaction (RT-PCR) assay for *Sox13* expression in TN, DP, and γδ thymocytes from WT mice. (**B**) Semiquantitative RT-PCR (sqRT-PCR) to determine relative amounts of *Sox13* mRNA in TN subsets from WT mice. TN1, $CD44^+CD25^-$TN; TN2, $CD44^+CD25^+$TN; TN3, $CD44^-CD25^+$TN. (**C**) RT-PCR for *Sox13* expression in $CD8^+TCR\beta^+$ and $CD8^+TCR\delta^+$ IELs, $CD4^+$ and $CD8^+$ αβ T cells, natural killer (NK) cells, and B cells. ND, not done. (**D**) sqRT-PCR for expression of indicated genes in DP and γδ T cells of WT mice. (**E**) sqRT-PCR for *Sox13* expression in DP and γδ thymocytes from $Tcr\beta^{-/-}$ mice. (**F**) Representative RT-PCR for *Sox13* and *Tcf1* expression in sorted single c-$kit^+CD44^+CD25^+$ TN2 thymocytes from WT mice ($n = 3$ independent experiments). The numbers above the lanes indicate individual clones. All 13 β-*actin*$^+$ single cells were *Tcf1*$^+$, whereas 13 of 28 β-*actin*$^+$ cells expressed *Sox13*.

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another recent documentation of the developmental heterogeneity at the TN2 stage (*11*), suggest that some lineage commitment has already occurred at the TN2 stage. To determine whether expression of *Sox13* marks a distinct subset of T precursors, we first established the frequency of *Sox13*-expressing cells among single TN2 cells. Whereas 100% of single TN2 cells expressed *Tcf1*, *Sox13* expression was detected in only 46% of the clones (Fig. 1F). This result indicates that *Sox13* expression is restricted to a subset of TN2 cells, and the determined frequency of *Sox13*-expressing TN2 cells correlates well with the frequency of bipotent or γδ-lineage–restricted adult TN2 cells, as determined by an in vitro assay (*11*). At the population level, a TN2 subset biased toward the γδ lineage expressed more *Sox13* than did the αβ-lineage–biased TN2 subset (fig. S2). These results support the prediction that *Sox13* expression marks cells more likely to become γδ T cells.

To assess the function of SOX13 in γδ- versus αβ-lineage development, we generated transgenic (Tg) lines that overexpressed *Sox13* in thymocyte precursors. αβ-lineage development was ablated in *Sox13*Tg embryonic day 17 (E17) fetuses as compared to littermate controls (LMCs) because DP cells were absent. In contrast, γδ cells developed normally (Fig. 2A). αβ T cell–lineage development remained impaired in adult *Sox13*Tg mice (4 to 8 weeks old), with a decrease in the proportion and number of αβ thymocytes when compared to LMCs. αβ DP cell numbers were reduced by 10- to 50-fold , depending on the age and founder lines (Fig. 2, B and C). Again, despite an increase in the percentage of γδ-lineage cells in *Sox13*Tg mice, the absolute number of these cells was comparable to that in the LMCs (Fig. 2, B and C).

Some HMG TFs have been implicated in the modulation of the cell cycle and in survival (*12*–*14*). Accordingly, there was a 2.5-fold decrease in bromodeoxyuridine (BrdU) incorporation in the TN cells in *Sox13*Tg mice as compared to LMCs (Fig. 2D). Because the vast majority of proliferating TN cells in the normal thymus are αβ-lineage preTCR$^+$ thymocytes, these results suggest that SOX13 inhibits the proliferative burst associated with the αβ-lineage TN-to-DP developmental transition. In addition, a greater than twofold increase in the frequency of apoptosis was observed in the DP and γδTCR$^+$ thymocytes of *Sox13*Tg mice relative to LMCs, as evidenced by the increased frequency of cells expressing Annexin V, an early marker of apoptosis (Fig. 2E), suggesting that SOX13 regulates cell survival. Concordantly, the deficit in DP thymocytes in *Sox13*Tg mice was partly reversed by the expression of an anti-apoptotic *Bcl2* transgene (fig. S3).

To test whether SOX13 is also required to impose a γδ-lineage molecular differentiation program on developing T cells, we compared global gene-expression profiles of sorted αβ-lineage DP thymocytes from *Sox13*Tg mice and LMCs. The majority of differentially expressed genes [70 or 57% of genes whose expression was decreased or increased, respectively, in *Sox13*Tg DP cells relative to the baseline wild-type (WT) DP cells] were also the genes differentially expressed between normal γδ and αβ DP thymocytes (Fig. 2F and fig. S4). The most notable gene among the differentially expressed genes was that encoding TCRγ, the prototypic γδ T cell–lineage marker, whose expression is normally silenced in αβ-lineage DP cells but was expressed in developing αβ cells in *Sox13*Tg mice. These findings suggest that enforced expression of SOX13 in αβ-lineage cells is sufficient to impose some aspects of the γδ-lineage molecular trait.

The phenotype of *Sox13*Tg mice resembled that of mice lacking T cell factor 1 (TCF1), another HMG TF essential for αβ T cell–lineage development (*12*, *15*, *16*). Indeed, the expression of the *Vγ2-Jγ1* gene rearrangement was evident in αβ T cells of *Tcf1*$^{-/-}$ mice (Fig. 3A), suggesting that one function of TCF1 is to repress TCRγ gene expression in αβ-lineage cells. In addition, TCF1 positively regulated αβ-

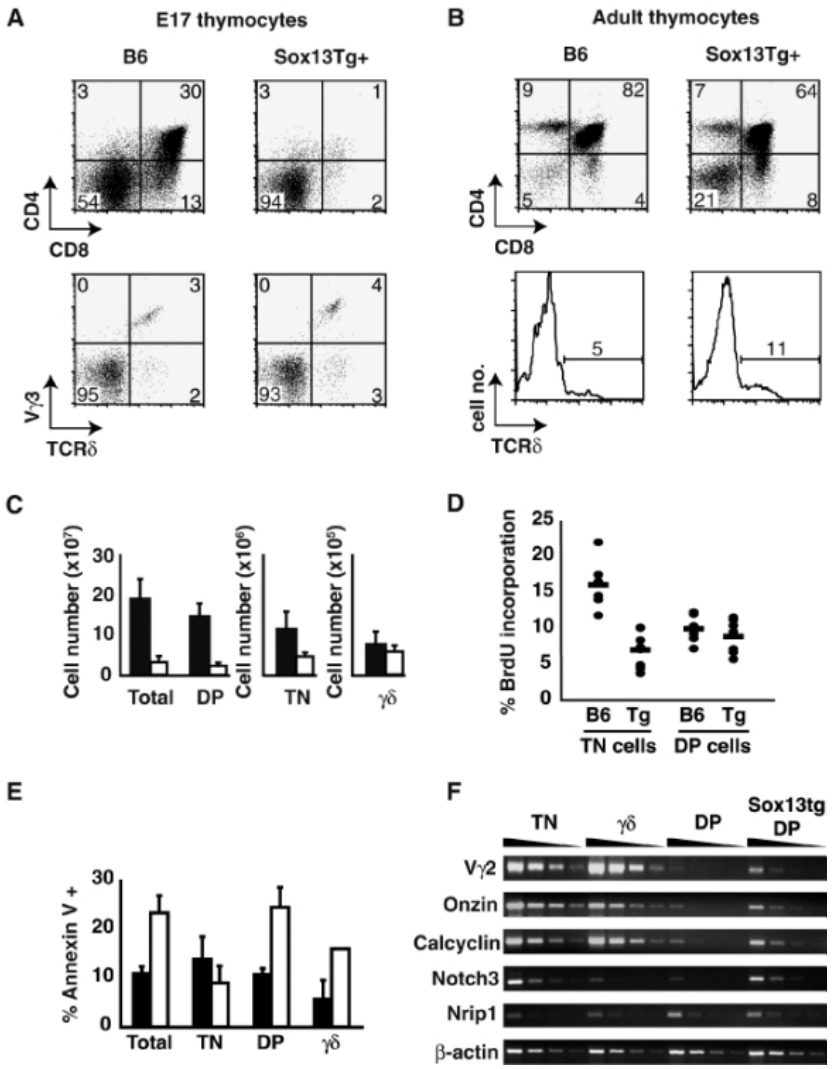

Fig. 2. SOX13 inhibits αβ-lineage development. (**A**) Thymocytes from E17 LMC and *Sox13*Tg fetuses were analyzed by flow cytometry for αβ- (CD4 and CD8) and γδ- (TCRδ and Vγ3) lineage markers. The profiles are representative of three litters of two Tg lines. (**B**) Thymocytes from adult LMC and *Sox13*Tg mice analyzed as in (A), except for TCRδ staining results, which indicate proportions in CD4$^-$CD8$^-$ thymocytes. Representative results of multiple mice (from two to more than seven) per Tg founder are shown. Seven of nine Tg founder lines showed defects in T cell development. (**C**) Numbers of thymocyte subsets from LMC (solid bars, n = 7) and *Sox13*Tg (open bars, n = 8) mice. Error bars indicate SD. (**D**) BrdU incorporation (1-hour pulse) in thymocytes from LMC and *Sox13*Tg mice. The circles represent BrdU incorporation in individual mice; the bars represent the mean value. (**E**) Cell-surface Annexin V on thymocytes from LMC (solid bars, n = 4) and *Sox13*Tg (open bars, n = 4) mice. Error bars indicate SD. (**F**) sqRT-PCR of TN and γδ thymocytes from WT mice and DP thymocytes from LMC and *Sox13*Tg mice to confirm select genes altered in *Sox13*Tg DP thymocytes identified by Affymetrix arrays (fig. S4).

lineage–specific *Cd4* gene expression (*17*), and *Sox13*Tg mice exhibited diminished CD4 expression on neonatal DP thymocytes (fig. S5), suggesting an antagonistic interaction between SOX13 and TCF1.

The activity of TCF1 is induced by canonical Wnt signaling by its association with the coactivator β-catenin (*12*, *18*, *19*). Some *Sox* genes have been shown to influence Wnt signaling (*20*, *21*), and we tested the possibility that SOX13 functions, in part, by antagonizing TCF1. 293T cells express TCF1 and signaling-competent β-catenin in sufficient amounts to activate transcription of a *Luciferase* reporter gene controlled by TCF binding sites (*22*). SOX13 inhibited this reporter gene activation in a dose-dependent manner (Fig. 3B). Further, SOX13 could down-modulate expression of *Ly49a,* a known in vivo TCF1 target gene (*23*), in EL4 T cells (Fig. 3C).

We next examined the mechanism by which SOX13 suppresses TCF1 function. In 293T cells, SOX13 was shown to bind full-length TCF1 (the p45 isoform), but not N-terminal truncated TCF1 (p33) that lacks the β-catenin interaction domain (Fig. 3D). Moreover, no evidence of direct SOX13 interaction with β-catenin or its related protein, γ-catenin, was detected (Fig. 3D). Hence, SOX13 is the first example of a lineage-specific Wnt/TCF1 antagonist that acts by directly

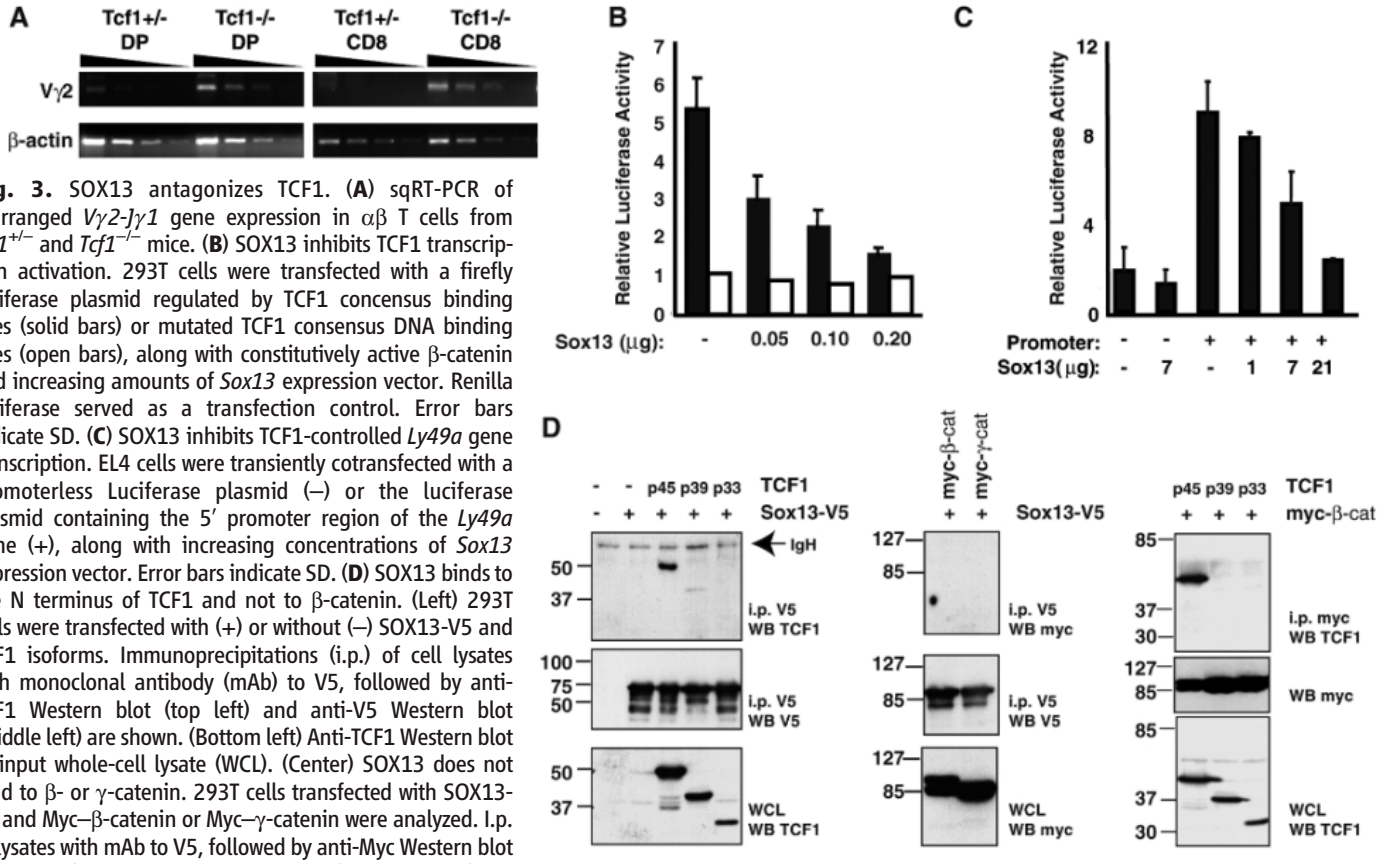

Fig. 3. SOX13 antagonizes TCF1. (**A**) sqRT-PCR of rearranged *Vγ2-Jγ1* gene expression in αβ T cells from *Tcf1*$^{+/-}$ and *Tcf1*$^{-/-}$ mice. (**B**) SOX13 inhibits TCF1 transcription activation. 293T cells were transfected with a firefly luciferase plasmid regulated by TCF1 concensus binding sites (solid bars) or mutated TCF1 consensus DNA binding sites (open bars), along with constitutively active β-catenin and increasing amounts of *Sox13* expression vector. Renilla luciferase served as a transfection control. Error bars indicate SD. (**C**) SOX13 inhibits TCF1-controlled *Ly49a* gene transcription. EL4 cells were transiently cotransfected with a promoterless Luciferase plasmid (–) or the luciferase plasmid containing the 5′ promoter region of the *Ly49a* gene (+), along with increasing concentrations of *Sox13* expression vector. Error bars indicate SD. (**D**) SOX13 binds to the N terminus of TCF1 and not to β-catenin. (Left) 293T cells were transfected with (+) or without (–) SOX13-V5 and TCF1 isoforms. Immunoprecipitations (i.p.) of cell lysates with monoclonal antibody (mAb) to V5, followed by anti-TCF1 Western blot (top left) and anti-V5 Western blot (middle left) are shown. (Bottom left) Anti-TCF1 Western blot of input whole-cell lysate (WCL). (Center) SOX13 does not bind to β- or γ-catenin. 293T cells transfected with SOX13-V5 and Myc–β-catenin or Myc–γ-catenin were analyzed. I.p. of lysates with mAb to V5, followed by anti-Myc Western blot (top center) and anti-V5 Western blot (middle center) are shown. The bottom center panel shows anti-Myc Western blot of WCL. (Right) β-catenin binds to p45 TCF1 in corresponding assays.

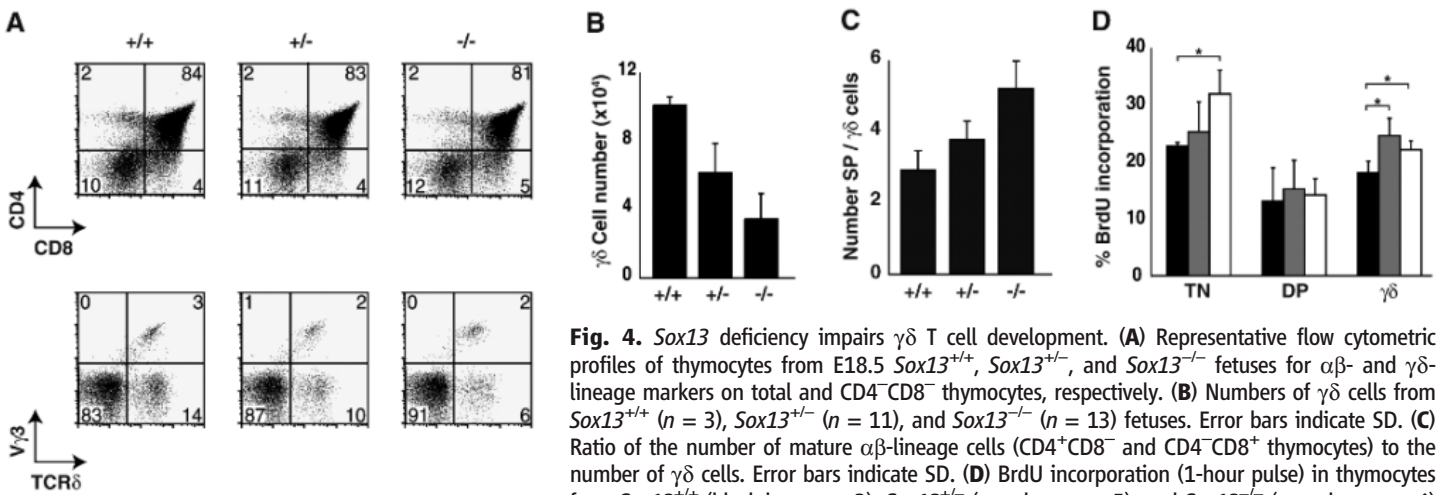

Fig. 4. *Sox13* deficiency impairs γδ T cell development. (**A**) Representative flow cytometric profiles of thymocytes from E18.5 *Sox13*$^{+/+}$, *Sox13*$^{+/-}$, and *Sox13*$^{-/-}$ fetuses for αβ- and γδ-lineage markers on total and CD4$^-$CD8$^-$ thymocytes, respectively. (**B**) Numbers of γδ cells from *Sox13*$^{+/+}$ (n = 3), *Sox13*$^{+/-}$ (n = 11), and *Sox13*$^{-/-}$ (n = 13) fetuses. Error bars indicate SD. (**C**) Ratio of the number of mature αβ-lineage cells (CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ thymocytes) to the number of γδ cells. Error bars indicate SD. (**D**) BrdU incorporation (1-hour pulse) in thymocytes from *Sox13*$^{+/+}$ (black bars, n = 3), *Sox13*$^{+/-}$ (gray bars, n = 5), and *Sox13*$^{-/-}$ (open bars, n = 6) E18.5 mice. * indicates $P < 0.05$. Error bars indicate SD.

binding and presumably sequestering TCF1 and/or modifying its activity.

To determine whether SOX13 is necessary for γδ T cell development, we generated *Sox13*-deficient mice. Because of as-yet-uncharacterized developmental abnormalities in postnatal $Sox13^{-/-}$ mice (fig. S6), we determined the effect of *Sox13*-deficiency during embryonic development where there are no overt developmental defects. No significant differences were observed in the proportions of αβ-lineage thymocytes at E18.5 (Fig. 4A). However, there was a gene dose–dependent decrease in the number and proportion of $\gamma\delta TCR^+$ cells in $Sox13^{+/-}$ and $Sox13^{-/-}$ mice as compared to LMCs (Fig. 4, A and B), resulting in a significantly increased ratio of the absolute number of mature αβ-lineage thymocytes relative to $\gamma\delta TCR^+$ thymocytes (Fig. 4C). This adverse effect of *Sox13* deficiency on γδ T cell development was evident despite the fact that $Sox13^{-/-}$ mice expressed *Sox5*, the closest relative of *Sox13* (*24*), whose expression pattern is identical to that of *Sox13* during mouse T cell development (fig. S6). In addition, $Sox13^{-/-}$ γδ and TN thymocytes exhibited significantly enhanced rates of proliferation (Fig. 4D), a corollary finding to reduced proliferation of TN thymocytes in *Sox13*Tg mice (Fig. 2E). These results indicate that the developing γδ thymocytes are extremely sensitive to the levels of SOX13 and that SOX13 is necessary for normal γδ T cell development.

Sox13 is the first true γδ-lineage–specific TF identified that is necessary for normal γδ T cell development. The heterogeneous expression of *Sox13* in TN2 cells, combined with the asymmetry in the developmental potential of the TN2 subset, indicates that some lineage separation had occurred before TCR rearrangement. The silencing of *Sox13* expression may constitute an initial step in the elaboration of the αβ-lineage molecular program. Although this conclusion does not preclude the influence of possibly distinct γδTCR and preTCR signaling modules in subsequently reinforcing or modifying the lineage-fate decision, the ability of SOX13 to antagonize TCF1 suggests that a simple on/off switch controlling the magnitude of Wnt/TCF1 signaling may be an early, critical regulator of the binary T cell–fate decision process.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5809/230/DC1
Materials and Methods
Figs. S1 to S6
References

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A Systems Approach to Measuring the Binding Energy Landscapes of Transcription Factors

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A major goal of systems biology is to predict the function of biological networks. Although network topologies have been successfully determined in many cases, the quantitative parameters governing these networks generally have not. Measuring affinities of molecular interactions in high-throughput format remains problematic, especially for transient and low-affinity interactions. We describe a high-throughput microfluidic platform that measures such properties on the basis of mechanical trapping of molecular interactions. With this platform we characterized DNA binding energy landscapes for four eukaryotic transcription factors; these landscapes were used to test basic assumptions about transcription factor binding and to predict their in vivo function.

Systems biology focuses on understanding the collective properties of biological networks; these networks in turn describe interactions between as many as thousands of unique elements. In recent years, knowledge of biological networks has grown dramatically, mainly because of the development and application of novel genomic (*1*–*3*) and proteomic methods (*4*–*8*) as well as bottom-up approaches such as genetic network engineering (*9*, *10*). But as network topologies are becoming well characterized, information about the elements comprising these networks has remained minimal and in the realm of low-throughput biology. In order to model and predict the behavior of these complex systems, the underlying interactions between elements will have to be quantitatively characterized.

Quantifying the affinities of molecular interactions is a considerable technical challenge. First, any particular biological interaction is governed by a large number of variables. Therefore, obtaining equilibrium dissociation constants requires one to perform dozens of assays as the concentrations of various components are systematically varied, increasing the number of measurements needed in an already logistically challenging problem. A second and more fundamental problem is the fact that many molecular interactions are transient and exhibit nanomolar to micromolar affinities, leading to rapid loss of bound material or little bound material in the first place. These factors are problematic for high-throughput methods such as yeast two-hybrid (*6*) and tandem affinity purification mass spectrometry (*4*, *5*), where transient interactions are frequently missed. Protein-protein (*11*) and protein-DNA binding microarrays (PBMs) (*12*–*14*) are especially susceptible because of their stringent wash requirements, causing rapid loss of weakly bound material. Protein arrays have been applied to quantify ligand–ErbB receptor inter-

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actions (*11*), with off rates determined by surface plasmon resonance to be on the order of 10^{-4} s^{-1} (*15*). PBMs have been applied in a semiquantitative manner to transcription factor (TF) motif analysis for high-affinity interactions, with off rates on the order of 10^{-3} s^{-1} (*12*–*14*).

We developed a high-throughput microfluidic platform (Fig. 1) capable of detecting low-affinity transient binding events on the basis of the mechanically induced trapping of molecular interactions (MITOMI), which eliminates the off-rate problem facing current array platforms and allows for absolute affinity measurements. We used MITOMI to map the binding energy landscapes of four eukaryotic TFs belonging to the basic helix-loop-helix (bHLH) family by collecting over 41,000 individual data points from more than 17 devices and covering titrations over 464 target DNA sequences. These binding energy topographies allowed us to (i) predict in vivo function for two yeast TFs, (ii) make a comprehensive test of the base additivity assumption, and (iii) test the hypothesis that the basic region alone determines binding specificity of bHLH TFs.

bHLH motifs represent the third largest TF family in eukaryotes and regulate a wide variety of cellular functions ranging from cell proliferation and development to metabolism (*16*). We studied isoforms A and B of the human TF MAX, which together with other bHLH members play a role in cellular proliferation and many cancers (*17*). We also studied the yeast TFs Pho4p and Cbf1p; the former regulates phosphate metabolism (*18*, *19*), whereas the latter regulates methionine synthesis as well as chromosome segregation, serving a structural role in the kinetochore (*20*–*22*). bHLH TFs generally bind to a consensus sequence of 5′-CANNTG-3′ (where N is any nucleotide) called enhancer box (E-box) (fig. S1, A and B), which was later found to be the second most conserved motif in higher eukaryotes (*3*). Members of the bHLH family show mid- to low nanomolar DNA binding affinities and have off rates above 10^{-2} s^{-1} for their consensus sequences (*23*, *24*), with orders of magnitude higher off rates for nonconsensus sequences. This transience makes the use of conventional microarrays impractical.

The TF binding energy topographies were measured with highly integrated microfluidic devices (*25*, *26*) containing 2400 independent unit cell experiments (Fig. 1, A and B). Each device is controlled by 7233 valves fabricated by multilayer soft lithography (MSL) (*27*) and programmed with a 2400-spot DNA microarray (*28*). The 2400 chambers are arranged into 24 rows addressed via a resistance equalizer (Fig. 1A); this ensures that flow velocities are equal across all rows, resulting in uniform surface derivatization and TF deposition. To avoid time-consuming cloning and protein synthesis and purification steps, we synthesized the TFs in situ via wheat germ–based in vitro transcription and translation (ITT). We designed a two-step polymerase chain reaction (PCR) method that generates linear expression–ready templates directly from yeast genomic DNA or cDNA clones (fig. S2). This approach allowed us to not only rapidly screen new TFs but also easily create and test structural chimeras.

We synthesized libraries of Cy5-labeled target DNA sequences that comprehensively cover the E-box motif and flanking bases by permuting up to four bases at a time (fig. S1C and data set 1). Dilution series for each target DNA sequence were spotted as microarrays with column and row pitches of 563 μm and 281 μm, respectively. These arrays were used to program the microfluidic devices by aligning

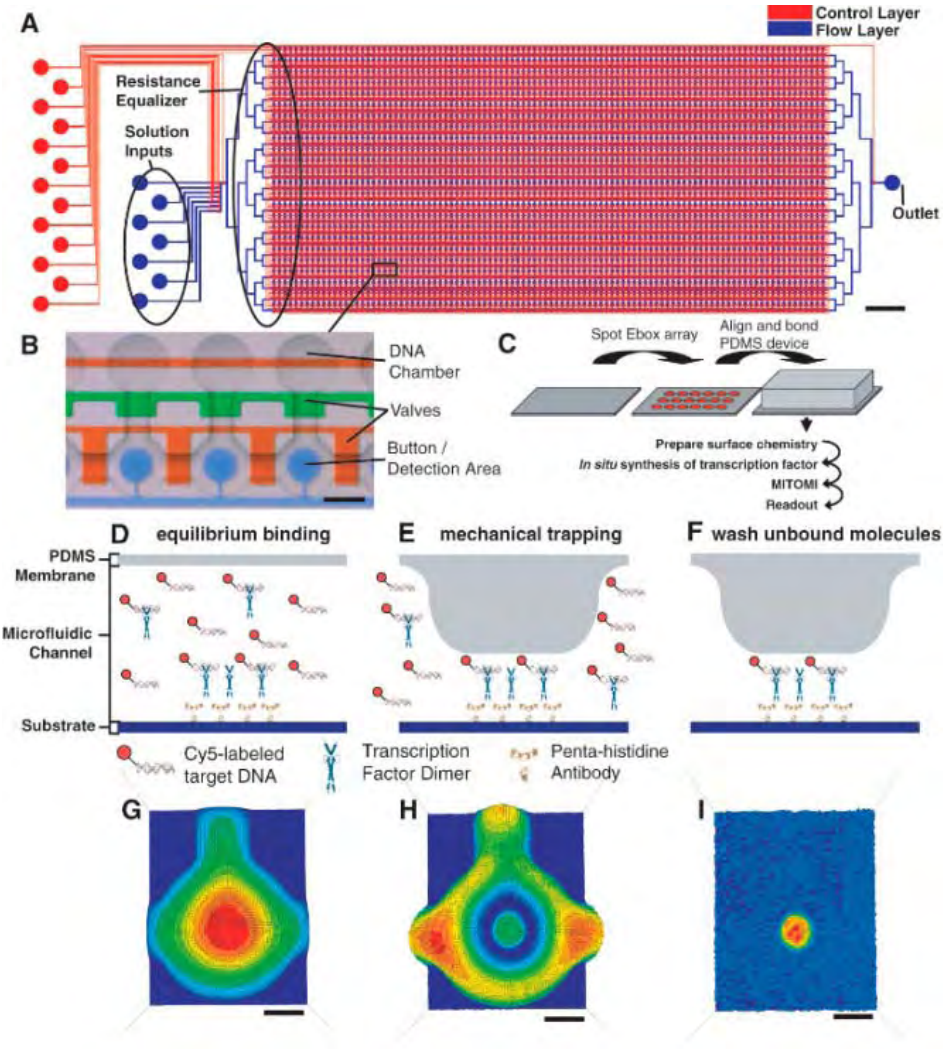

Fig. 1. (**A**) Design drawing of the microfluidic device. Red and blue lines represent control and flow channels, respectively. The device contains 2400 unit cells controlled by 7233 valves (scale bar indicates 2 mm). (**B**) Optical micrograph of three unit cells. Control channels are filled with colored food dyes for visualization. Each unit cell consists of a DNA chamber aligned to a microarray spot and a detection area. The valves shown in green control access to the DNA chambers, whereas the orange valves compartmentalize the unit cells. The button membrane is shown in blue and represents the area where detection takes place (scale bar, 150 μm). (**C**) Schematic outline of the approach. First, a microarray of target DNA sequences is spotted onto an epoxy slide. The microarray is then aligned and bonded to a microfluidic device. Next, the necessary surface chemistry is prepared, followed by in situ synthesis of TF and detection of interactions by MITOMI. (**D** to **F**) Schematic of the process of MITOMI. The gray structure at the top of each panel represents the deflectable button membrane that may be brought into contact with the glass surface (blue). (D) His_5-tagged TFs are localized to the surface, and TF-DNA binding is in a steady state. (E) The button membrane is brought into contact with the surface, expelling any solution phase molecules while trapping surface-bound material. (F) Unbound material not physically protected is washed away, and the remaining molecules are quantified. (**G** to **I**) Fluorescent intensity maps of target DNA concentration. (G) to (I) correspond to the states schematically shown in (D) to (F) (scale bars, 50 μm).

each spot to a unit cell. The ability to program devices with microarrays simplifies the microfluidic infrastructure and increases unit cell density. The use of microarrays for device programming is highly modular because any soluble substance or suspension may be arrayed, and it provides an elegant and efficient solution to the world-to-chip interface problem. Approximately attomoles of DNA and TF are required for each data point.

Each unit cell is controlled by three micromechanical valves (*27*) as well as a "button" membrane (fig. S3) used for surface derivatization and MITOMI (Fig. 1, B to I, and fig. S4). When the button is actuated, it physically blocks a 60-μm circular area on the slide, preventing molecules from entering or leaving that part of the surface. The contact area can be precisely modulated by choice of button diameter and closing pressures (fig. S3). During surface derivatization, a circular area is masked with the button, while the rest of the surface is passivated with biotinylated bovine serum albumin. When the button is released, the previously protected circular area is specifically functionalized with biotinylated antibody against His_5 (fig. S4, C to G). After surface patterning, the device is loaded with wheat germ–based ITT mixture containing linear DNA template coding for the TF to be synthesized, and each unit cell is isolated by closing a set of micromechanical valves. The device is incubated at 30°C for 90 min to complete TF synthesis, solvation of target DNA, and equilibration of TF and target DNA (fig. S4, H and I). After the incubation period, MITOMI is performed by again actuating the button membrane and trapping surface-bound complexes (Fig. 1, D to I, and fig. S4, J to L). Initial contact of the membrane with the surface occurs medially and extends radially outward. Radial closure prevents solvent pockets from forming between the two interfaces and effectively creates zero dead volume while preserving the equilibrium concentrations of the molecular interactions to be detected. The trapped molecules are subsequently quantified with a DNA array scanner (fig. S5). Device characterization and control experiments are described in the Supporting Online Material (figs. S6 to S8) (*26*); we determined the lower limit of detection to be $K_d \approx 18$ μM and established a global measurement error of 19% (fig. S9).

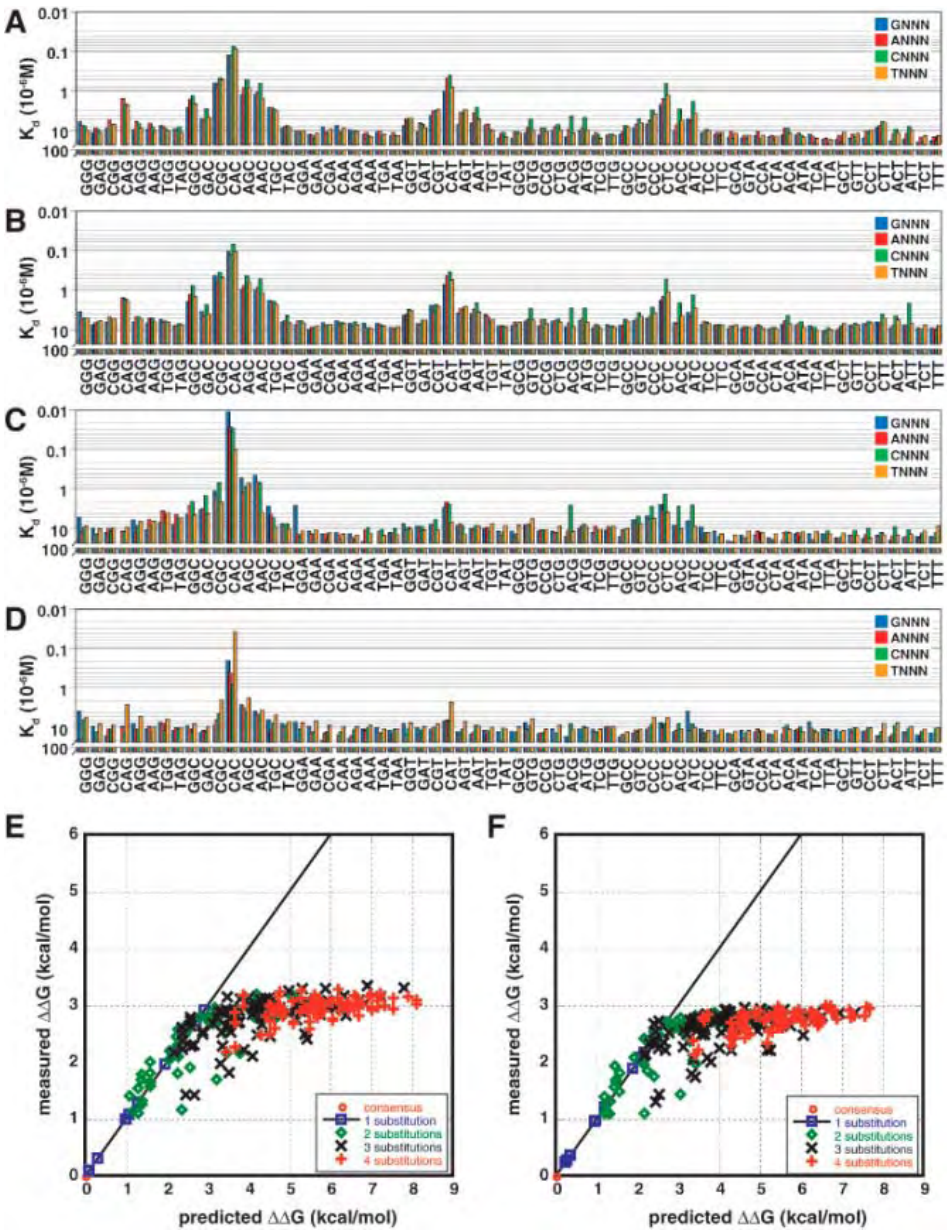

Fig. 2. Binding affinities of C-terminally tagged TFs MAX iso A (**A**), MAX iso B (**B**), Pho4p (**C**), and Cbf1p (**D**) to all sequence permutations of N_{-4} to N_{-1}. Sequences N_{-3} to N_{-1} are plotted on the category axis, with the fourth base, N_{-4}, displayed as clusters of four columns per category. (**E** and **F**) Comparisons of predicted changes in the Gibbs free energy ($\Delta\Delta G$) against measured values for MAX isoforms A and B are shown, respectively. All predicted values were calculated from PWMs assuming base independence.

Our measurements agree with previous reports that the optimal binding sequence for all four TFs is CACGTG for N_{-3} to N_3 (Fig. 2, A to D, and figs. S10, A to D, and S11). We measured consensus binding affinities of 67.0 nM, 73.1 nM, 11.1 nM, and 16.6 nM for MAX isoform A, MAX isoform B, Pho4p, and Cbf1p, respectively. The binding affinity of MAX to a slightly different sequence has been measured independently and is in agreement with our result for that sequence (*24*). Each binding energy landscape exhibits topographic fine structures, such as affinity spikes for sequences with a one-base spacer between the two half sites (CACGGTG for example) as well as consensus neighbors CATGTG, CTCGTG, and CAGGTG. These fine structures often lie in the low-affinity regime (with off rates on the order of 2 to 20 s^{-1}) and have thus far not been observed with other methods. The binding energy landscapes for both MAX isoforms are more rugged than the landscapes of Pho4p and Cbf1p, showing strong affinities for consensus neighbors, whereas Pho4p and Cbf1p are singularly specific for the E-box consensus. These differences in topography are intriguing, because crystal structures of truncated versions of MAX and Pho4p show that both TFs make essentially the same base-specific contacts (*29*, *30*). Therefore, similar base-specific contacts give rise to recognition of the same consensus sequence but not necessarily to similar overall binding topographies.

Informatic methods for TF binding motif discovery often rely on ad hoc hypotheses such as the additivity assumption, which

posits that the energetic or informatic role of each base in a given motif is independent of the identity of neighboring bases. Evidence from small data sets contradicts this assumption (*31*, *32*), but there is an ongoing debate on the importance of the observed non-independence (*33*). Our data on the absolute binding affinities of the MAX isoforms to all possible sequence permutations of one E-box half site plus a flanking base, or 256 sequences in total, allowed us to determine the extent of interdependence between individual base contacts. First, we generated a position weight matrix (PWM) for each isoform, consisting of changes in the Gibbs free energy for all 16 possible single-base substitutions. These PWMs were then used to calculate binding affinities to all 256 sequences. Because PWMs contain no information on higher-order interactions, the predicted affinities reflect an assumption of completely independent base contacts. Comparing our experimentally determined values with the predictions showed that only a small subset of values agreed (Fig. 2, E and F, and fig. S10, E and F). PWMs fail to predict low-affinity binding, because almost no sequences above 2.5 kcal/mol agreed with measured values (defined as lying within 2σ or ±0.4 kcal/mol of the measured value). This is mostly due to the increasing role of non-specific interactions, for which failure of the PWM is not surprising. More importantly, PWMs also fail for higher affinities, predicting only 44% of all sequences below 2.5 kcal/mol. The PWMs predicted only 56%, 10%, and 0% of all double, triple, and quadruple substitutions, respectively. The consequences of these results are twofold. First, for gene discovery applications PWMs will give low false positives but more potential false negatives or missed genes. Second, PWMs are not sufficient for more detailed computational approaches to systems biology that seek to understand the stochastic dynamics of TF binding.

To address the question of how Pho4p and Cbf1p serve distinct biological functions while recognizing seemingly identical consensus motifs, we measured the extent to which these TFs recognize bases flanking the E-box consensus. Current experimental and bioinformatic methods (*1*, *7*, *34*, *35*) failed to show definite differences in sequence recognition between these two TFs (CACGTGsG and rTCACGTG for Pho4p and Cbf1p, respectively, where r is any purine and s is G or C). We measured all possible permutations of three flanking bases 5′ as well as 3′ of the consensus sequence in order to determine how far base-specific recognition extends. The results show that Pho4p specifically recognizes two flanking bases, extending the consensus motif to a palindromic decamer of 5′-CCCACGTGGG-3′ (Fig. 3A and fig. S12, A and B). For Cbf1p, the flanking base recognition profile differed drastically from Pho4p, preferring GT as $N_{-5}N_{-4}$ (Fig. 3B) and the palindrome AC for N_4N_5 (fig. S12, C to D). Furthermore T and C were preferred for N_6 (A and G in terms of N_{-6}), extending Cbf1p sequence recognition from the initial hexamerous E-box motif to a dodecamer of 5′-[A/G]GTCACGTGAC[T/C]-3′.

On the basis of structural (*29*, *30*) and biochemical data, it is widely assumed that the sequence-specific binding of bHLH TFs is determined entirely by the basic region. We assessed whether the basic region itself is sufficient to produce the observed flanking base sequence specificity by cloning the basic regions of Pho4p and Cbf1p into the MAX isoform B backbone. The results show that, despite the existence of a few intriguing sequence outliers, the basic region itself is sufficient to transform the original MAX isoform B recognition pattern to patterns resembling Pho4p and Cbf1p (Fig. 3D).

Last, we asked whether the binding energy landscapes for Pho4p and Cbf1p are sufficient to predict which genes these TFs physically bind and therefore likely regulate. We applied a simple in silico model based on calculating a probability of occupancy (P_{occ}) (*36*, *37*) for each regulatory sequence of 5814 yeast genes and obtained 38 and 24 genes bound by Pho4p and Cbf1p, respectively. (Fig. 4, A and B, and data sets 2 and 3). We then tested whether these gene sets were significantly enriched for functions related to Pho4p (phosphate metabolism) and Cbf1p (methionine metabolism and chromosome segregation). The Pho4p data set showed significant enrichment in genes functioning in ionic homeostasis and phosphate metabolism (table S1), with 60% of the genes in the data set functioning in phosphate metabolism (37%), ionic homeostasis (13%), and vacuoles (10%) (Fig. 4A). Chromatin immunoprecipitation (ChIP)–chip and microarray experiments determining Pho4p-regulated genes have only 18% agreement; our data set includes all of these overlapping genes and has 40% agreement with at least one of the other data sets (Fig. 4E). For Cbf1p, the functional enrichment returned categories mainly involved in cell cycle and cell growth (table S1), implying that Cbf1p functions in chromosome segregation. Of Cbf1p-regulated genes, 30% are involved in chromosome structure, and another 25% are involved in budding (Fig. 4B). Only two genes (8%) regulate methionine synthesis. We also found three genes (*STE20*, *GAL2*, and *DRS2*) that

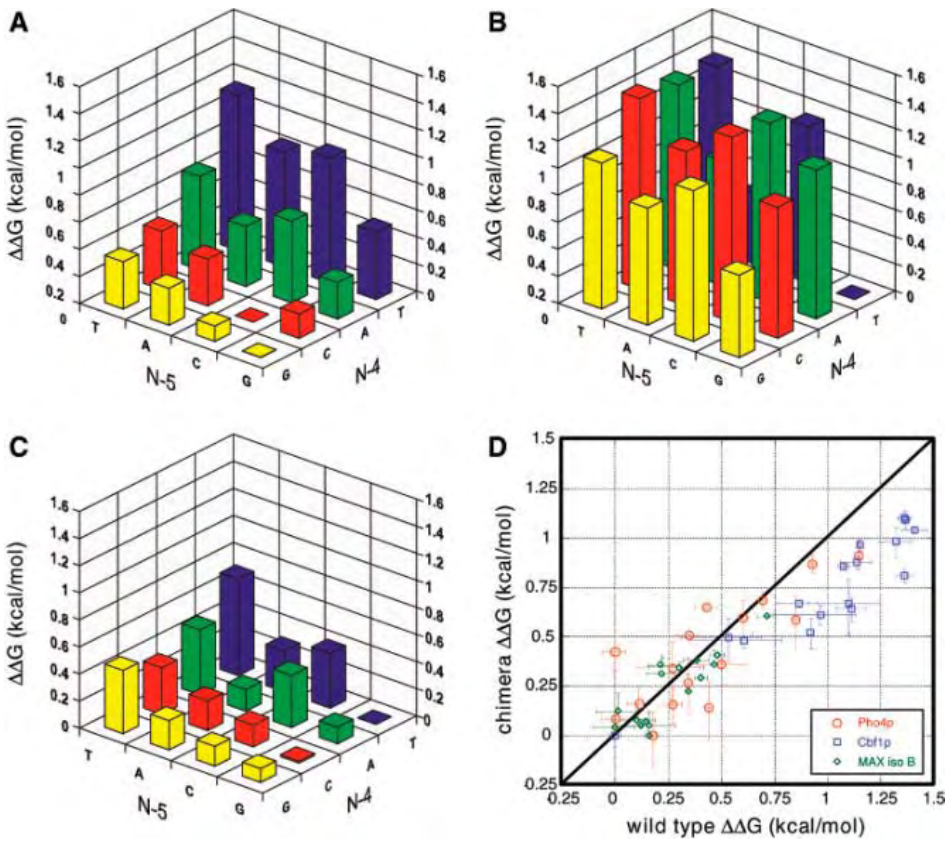

Fig. 3. $\Delta\Delta G$ values of all permutations of the two flanking bases $N_{-5}N_{-4}$ for Pho4p (**A**), Cbf1p (**B**), and MAX iso B (**C**). (**D**) Comparison of the $\Delta\Delta G$ values of the wild-type proteins shown in (A) to (C) with basic region chimeras in which the basic region of MAX isoform B was replaced by the basic regions of Pho4p, Cbf1p, and MAX iso B. The average of two experimental values is plotted, with the difference shown by the error bars.

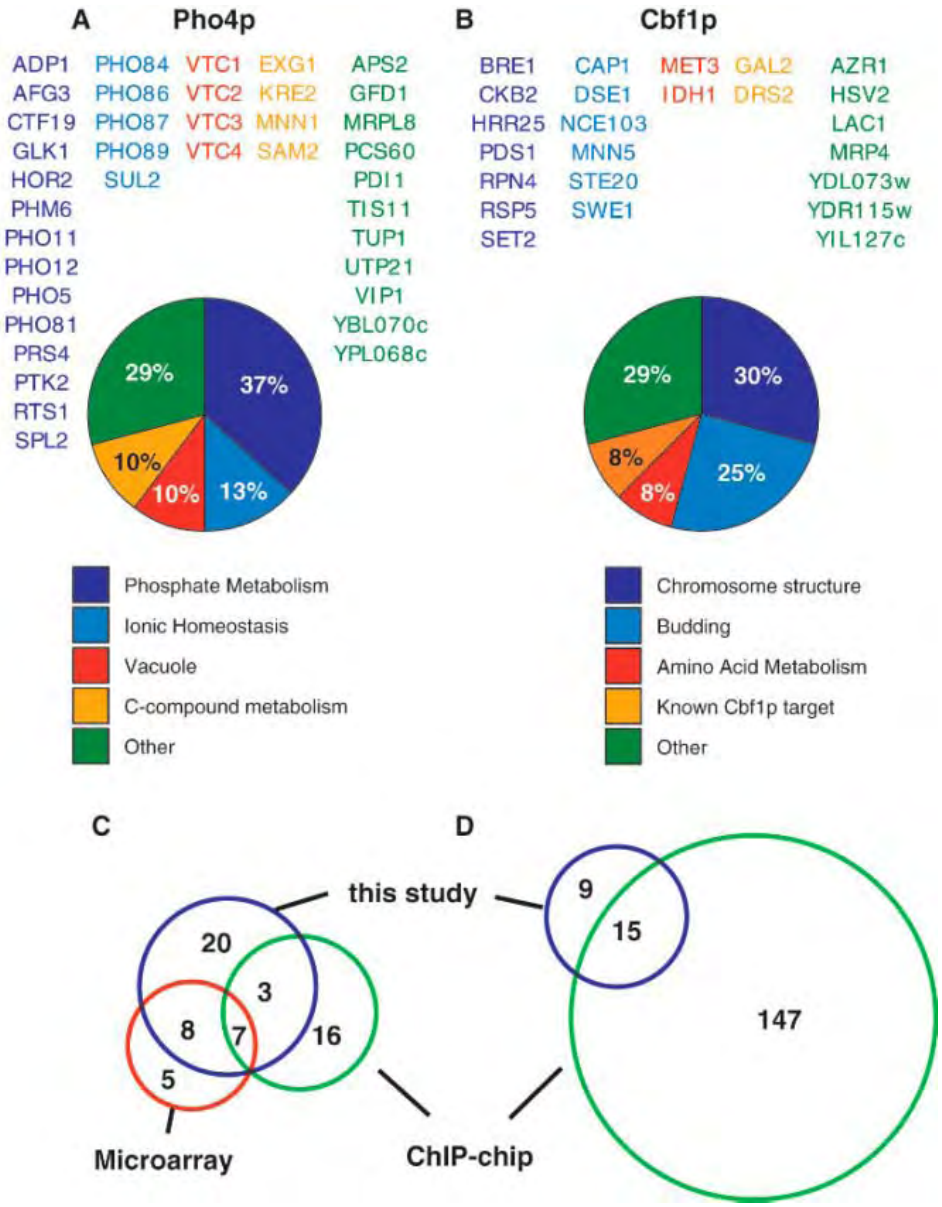

Fig. 4. In vivo function prediction for Pho4p and Cbf1p. (**A** and **B**) Genes with regulatory sequences determined to be bound by our in silico method. All genes shown here have a P_{occ} of above 0.2 and a sensu stricto conservation score of 25% or above. Pie charts show the functional distribution of the gene sets. (**C** and **D**) Venn diagrams comparing our predicted gene sets to gene sets determined with use of expression microarrays and ChIP-chip.

had previously been shown to exhibit Cbf1p-dependent chromatin remodeling (*20*). We predict that Cbf1 regulates a much smaller set of genes than ChIP-chip experiments do, but there is good agreement because more than 60% of the genes in our prediction were also found by ChIP-chip (Fig. 4F). We have thus shown that augmenting the consensus binding motifs with the new flanking bases revealed by the free energy landscapes results in concrete biological predictions that differentiate the function of Pho4 and Cbf1 and have broad agreement with the biological literature.

For two yeast TFs we have successfully predicted biological function by combining purely in vitro biophysical measurements with informatic knowledge of the organism's genome. As the theoretical and experimental tools of systems biology become more mature, this situation may become the rule rather than the exception, and it is worth considering whether there is any fundamental limit on the ability to predict biological behavior from in vitro measurements.

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Materials and Methods
Figs. S1 to S12
Table S1
References
Data sets 1 to 3

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A Large-Scale Deforestation Experiment: Effects of Patch Area and Isolation on Amazon Birds

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As compared with extensive contiguous areas, small isolated habitat patches lack many species. Some species disappear after isolation; others are rarely found in any small patch, regardless of isolation. We used a 13-year data set of bird captures from a large landscape-manipulation experiment in a Brazilian Amazon forest to model the extinction-colonization dynamics of 55 species and tested basic predictions of island biogeography and metapopulation theory. From our models, we derived two metrics of species vulnerability to changes in isolation and patch area. We found a strong effect of area and a variable effect of isolation on the predicted patch occupancy by birds.

Tropical forests are among the world's most threatened ecosystems, which are under severe pressure by human activities, primarily deforestation (*1*). Deforestation reduces forest area and frequently isolates remaining forest patches. Island biogeography (*2*) and metapopulation (*3*) theories predict the effects of reduced area and increased isolation on rates of species extinction and colonization and thus on species occurrence. However, strong tests of these predictions are scarce. Most published inferences about the effects of area and isolation are based on patterns of species occurrence, rather than directly on the dynamic rate parameters (such as extinction and colonization) that produce these patterns. Multiple plausible hypotheses can be developed to explain any such pattern, and pattern-based analyses thus produce weak inferences (*4*). The few studies that have focused on patch-occupancy dynamics follow observational, rather than experimental, designs at small spatial and temporal scales, and they fail to deal adequately with detection probabilities: the fact that species may be present at a site yet go undetected.

The Biological Dynamics of Forest Fragments Project (BDFFP) was initiated to test the effects of forest destruction at the patch level in a tropical ecosystem (*5*). The study, located in the central Brazilian Amazon, involved a reduction in forest area, which was predicted to increase the probabilities of local extinction of species, and isolation of remnant forest patches, which was predicted to reduce species colonization probabilities and increase extinction (*2*, *3*, *6*). The combined effects of both factors are predicted to reduce patch occupancy (*2*, *3*). We tested these predictions using data from the diverse bird community while separating inferences about area and isolation, in contrast to more usual approaches that confound these two effects as "fragmentation" (*7*). The data are of an experimental nature on relevant geographical and temporal scales, and our analysis treats detection probabilities explicitly.

A mist-netting program monitored understory birds in 23 primary-forest patches for 13 years (*5*). All patches were initially in continuous forest, but 11 of them were subsequently isolated by ranchland (two of which are depicted in Fig. 1). Patches were set in size classes of 1, 10, 100, 500, and 600 ha, with the largest isolated patch at 100 ha (table S1). The forest was cleared after monitoring began, permitting inference about the processes of extinction and colonization in isolated and continuous forest patches of different sizes (*8*).

We used patch-occupancy models (*9*) to test a priori hypotheses about the influence of patch area and isolation on occurrence dynamics of 55 well-sampled bird species. The models contain four kinds of parameters: initial occupancy (ψ_1), local extinction probability (ε), local probability of colonization (γ), and probability of detection given presence (p). We limited our model set to plausible a priori hypotheses about the processes of detection, colonization, and local extinction, rather than conducting exploratory analyses with a larger model set including various combinations of potential covariates (table S2). ψ_1 is a free parameter and is never related to a covariate. p is a function of mist-netting effort and takes different values for different species. We predicted that ε would be related to patch area and isolation in one of three ways: a multiplicative function of patch size and isolation (full interaction model), an additive function of the same two variables, and a function of patch size alone. γ should be related to isolation, perhaps as modified by regrowth of matrix habitat because some ranchland was abandoned. Colonization is modeled as a function of isolation, regrowth, and the "year 1 effect"—increased colonization by displaced birds immediately after deforestation—taken in five additive combinations. Isolation is a binary variable. We thus fitted $3 \times 5 = 15$ different models to each species using a maximum-likelihood approach in the program PRESENCE (*10*). Models were ranked by Akaike's information criterion (AIC) and AIC weight w_j for model j (*11*). We predicted that patch size, independently of isolation, should have a negative effect on ε (*2*, *3*). Isolation, independently of size, should have a positive effect on local extinction via the reduction of the "rescue effect" (*6*).

We also asked three expert neotropical ornithologists to classify the study species into two categories of dispersal ability (low and high). Isolation, through its effects on colonization and local extinction, should be more important for models of poor dispersers than for models of good dispersers. We expected forest regrowth to be more important for poor dispersers than for good dispersers. Among the variables that affect ε and γ, we predicted that dispersal ability would be the most relevant in the species' responses to deforestation. Other variables may also be relevant, but their predicted effects are typically based on their relationship to dispersal ability. Thus, rather than testing the relevance of various species classification schemes, we focus here on our a priori prediction about the relevance of dispersal ability.

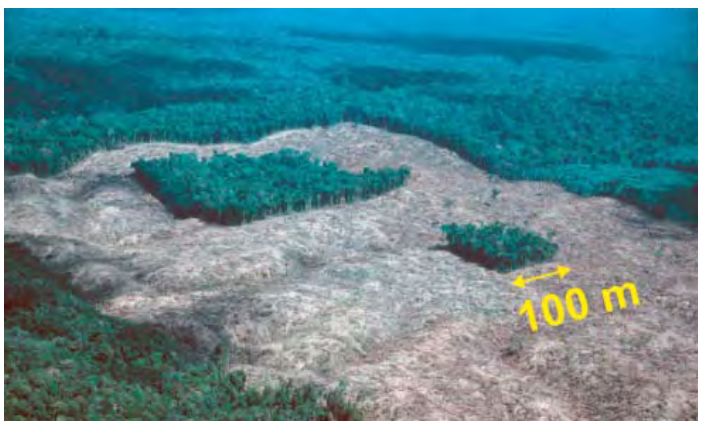

Fig. 1. Two isolated fragments at Fazenda Dimona, Brazil. This aerial photograph of a 10-ha and a 1-ha fragment was taken shortly after isolation. Gaps between the fragment edge and the continuous forest are less than 1 km wide.

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Our analysis follows three steps, all of which are based on fitting models for each species. First, we ask what covariates appear in the top-ranking models of each species. Second, we focus on the single model that fits best across species and examine the signs and magnitudes of covariate effects (slope parameters). Finally, we select one best-fitting model per species and draw inferences based on the estimated local extinction and colonization parameters.

Patch isolation appears as a covariate of colonization and/or local extinction in high-ranking models ($w_j > 0.2$) of nearly all, but not all, species (Table 1 and table S3). Patch size, regardless of isolation, seems sufficient to explain the observations on three exception species: *Geotrygon montana*, *Dendrocolaptes certhia*, and *Hypocnemis cantator*. Fifteen species have high-ranking models with an effect of isolation only on local extinction, and five species have high-ranking models with that effect only on colonization. Regrowth enters high-ranking models in two-fifths of the species. Contrary to our expectation, we cannot reject the null hypothesis that regrowth enters high-ranking models of high- and low-dispersal species in the same proportions (one-tailed z test, $P = 0.33$). Likewise, there is no evidence to sustain the prediction that isolation would appear more frequently in high-ranking models of low-dispersal species (one-tailed z test, $P = 0.97$). Inferences are qualitatively the same if high-ranking models are redefined as having $w_j > 0.1$ or $w_j > 0.25$.

Comparison of estimated slope parameters for area and isolation across species is facilitated by use of a single model, so we focus on model 6 (table S2 and Fig. 2), which has the highest average w_j across species. Model 6 hypothesizes fixed colonization and an additive effect of size and isolation on local extinction. There is a negative effect of patch size on ε: For all species, larger plots have lower ε values. Isolation shows more variable results, with 36 of 55 species showing a positive slope. As predicted, the effect of isolation on local extinction is positive more often than negative (one-tailed z test, $P < 0.01$), but for roughly one-third of the species, slope estimates are very close to zero (or even negative). We found no evidence to suggest that poor dispersers show positive isolation effects on extinction more often than do good dispersers (one-tailed z test, $P = 0.49$). Confirming previous inferences (*12*, *13*), all obligate ant-followers (*Pithys albifrons*, *Gymnopithys rufigula*, and *Dendrocincla merula*) and many mixed-species–flock attendants (*14*) showed evidence of a positive effect of isolation on local extinction, despite their high dispersal ability (table S3). Model 6 concentrates the effects of size and isolation on only one of the dynamic rate parameters (local extinction). The support for this model suggests that many species would be equally good at colonizing isolated and continuous forest patches. However, our ability to infer changes in colonization may be limited by the greater opportunity to see extinctions than colonizations in our data (*8*). It is very possible that more data may lead to more evidence of effects on colonization.

By selecting the best-fitting model for each species, we can account for the effect of regrowth and for the possible interaction between the effects of size and isolation on local extinction (table S4) (*8*), which are two aspects that model 6 did not address. We select only from the subset of 10 models that includes the covariate isolation affecting local extinction to ensure that we always estimate an effect of isolation on occupancy parameters, no matter how small. For each species-model combination, our analyses provided estimates of local extinction and colonization, expressed as functions of the pertinent covariates (fig. S1). These two parameters combine into a single population-dynamic metric that predicts equilibrium patch occupancy $\psi^*_{i_s}$, where i denotes isolation and s denotes patch size (*3*, *8*). Figure 3 illustrates how $\psi^*_{i_s}$, estimated for isolated and nonisolated patches of different sizes, may reflect different responses to landscape change. From the ensemble of each species' $\psi^*_{i_s}$ values, obtained from the best-fitting model for the species, we compute two metrics that separate specific effects of patch size and isolation. Species that have large territories or are otherwise sparsely distributed should have relatively low $\psi^*_{i_s}$ values in small patches of continuous forest. Accordingly, we formulate an index of area sensitivity A (Fig. 3C) as the relative reduction in $\psi^*_{i_s}$ from the largest to the smallest continuous-forest (cf) patch:

$$A = 1 - \frac{\psi^*_{cf_1}}{\psi^*_{cf_{100}}} \quad (1)$$

with the numerical subscript denoting patch size (in hectares). The uncommon black-throated antshrike (*Frederickena viridis*), a forest-interior antbird with narrow habitat requirements, exemplifies a species that is highly sensitive to area (Fig. 3, D to F). Poor colonizers that rarely cross open areas, or species that do not survive well in isolation, should have relatively low $\psi^*_{i_s}$ values in isolated patches. Thus, we measure vulnerability to isolation I (Fig. 2C) as the relative reduction in $\psi^*_{i_s}$ from 1-ha continuous-forest to 1-ha isolated (isol) patches:

$$I = 1 - \frac{\psi^*_{isol_1}}{\psi^*_{cf_1}} \quad (2)$$

The white-chinned woodcreeper (*D. merula*), a bird that forages by following swarms of army ants, is highly vulnerable to isolation but not sensitive to area (Fig. 3, A to C).

We use approximate 95% confidence intervals (Fig. 4) to assess whether each metric is significantly different from zero. In agreement with results based on slope parameters and w_j, a substantial proportion (29/54) of species is not significantly vulnerable to isolation. There is a

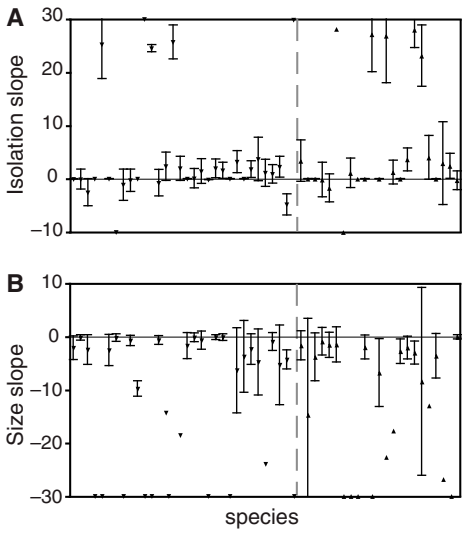

Fig. 2. Slope parameter estimates for the effect of isolation (**A**) and patch size (**B**) on local extinction, as according to model 6. Low- and high-dispersal species (table S3) appear on the left and right sides of the dashed line, respectively. Error bars indicate approximate 95% confidence intervals, assuming normally distributed parameter estimates. Points without confidence intervals on the upper or lower edges of the plot indicate point estimates beyond the limits of the y axis. Confidence intervals could not be estimated for all points.

Table 1. Contribution of isolation and regrowth covariates to high-ranking models of 50 species, grouped by dispersal ability. Isolation is a binary variable ("1" for isolated patches and "0" for otherwise). Regrowth counts the number of years since isolation. Five species had no models with $w_j \geq 0.2$.

	Number of species			
Does the isolation or regrowth covariate enter any part of a model with $w_j \geq 0.2$?	**Isolation**		**Regrowth**	
	Yes	**No**	**Yes**	**No**
Dispersal ability of species				
Low	25	3	11	17
High	22	0	9	13

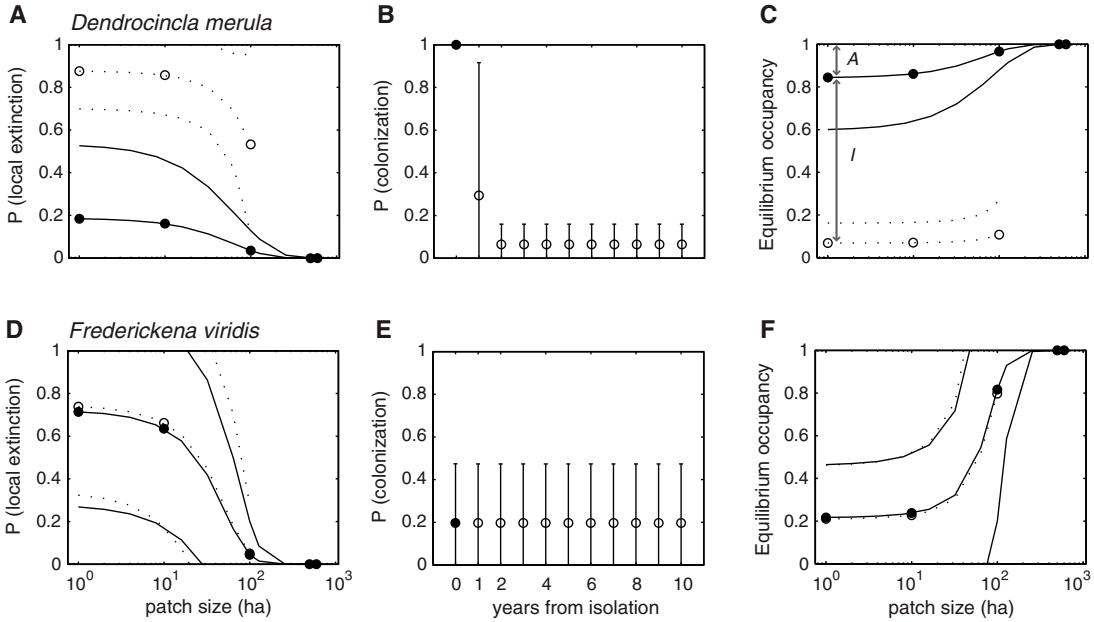

Fig. 3. Example of patch-occupancy parameters estimated by the best-fitting models for two species. Solid and open circles show estimates for continuous forest and isolated patches, respectively; error bars and lines without symbols show 95% confidence intervals. $\psi^*_{i_s}$ (**C** and **F**) is a function of ε (**A** and **D**) and γ (**B** and **E**). *D. merula* [(A) to (C)] shows a marked effect of isolation both on local extinction and on colonization and a stronger effect on $\psi^*_{i_s}$. Estimates for *F. viridis* [(D) to (F)] vary mostly with patch area. Arrows in (C) illustrate two metrics reflecting use of space: *A* and *I*.

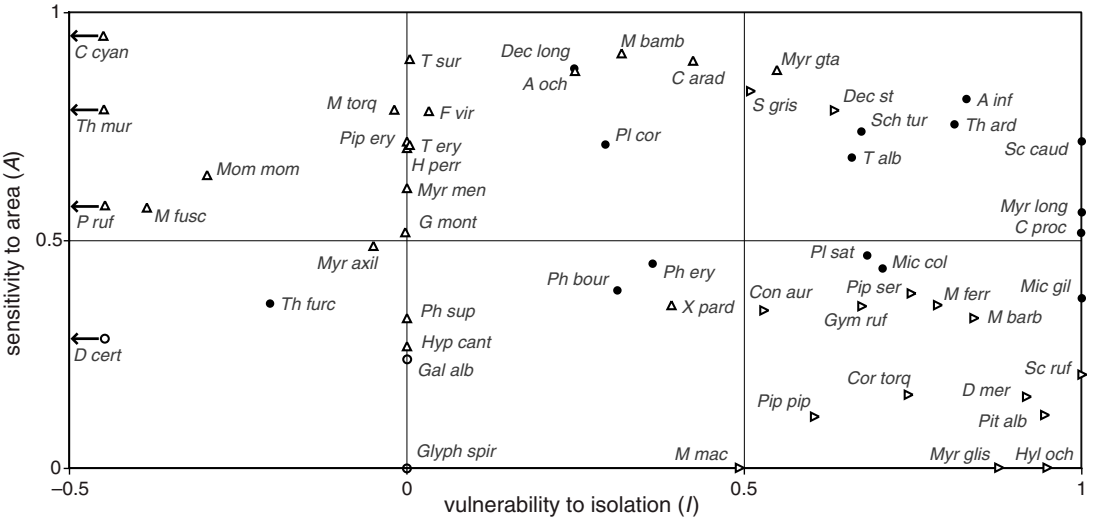

Fig. 4. Values of *A* and *I* for 54 species grouped by uncertainty over the two metrics (table S4). Open and solid circles indicate that the approximate 95% confidence intervals of both metrics or of neither metric overlap zero, respectively. Upward and rightward pointing triangles indicate that only the confidence interval of *A* or *I* does not overlap zero, respectively. Symbols with arrows indicate point estimates outside the plotted range of the *x* axis.

higher proportion (36/54) of species with area sensitivity that is significantly different from zero, with two-thirds of these species not being significantly vulnerable to isolation. This result suggests that many species fail to occur in small isolated patches, not because they succumb to the effects of isolation in reducing any rescue effect but because they rarely occupy any small patch (even in continuous forest) just because of their pattern of space use. Management aimed at curbing the effects of isolation (e.g., corridors and edge protection) will have reduced effectiveness for those species that are highly sensitive to area.

Our finding that local extinctions are always more probable in small than in large patches is predicted by demographic theory and is evident from the slope parameters of model 6. The role of isolation is less obvious: A predominantly positive but variable effect of isolation on local extinction under model 6 must be qualified by the many low and uncertain estimates of *I*. Our knowledge of the dispersal abilities of species in undisturbed conditions does not explain the variation in the signs of the slope parameters or in the contributions of the isolation and regrowth covariates to high-ranking models. Either we do not know enough about dispersal abilities—a notion that is widely supported by neotropical ornithologists—or those abilities change in disturbed landscapes to the extent that we cannot use them to predict occupancy parameters under disturbance.

Deforestation results in forest-area reduction and isolation of remaining forest patches, and both factors affect occupancy dynamics. This study shows the generality of these effects by showing that they apply to tropical forest landscapes in addition to the temperate landscapes from which most previous inferences have come. The interspecific variation in the effects of isolation on the studied tropical bird community is reminiscent of similar variation reported for avian communities in temperate forests of North America (*15*). Both the North American study and our more realistic modeling approach report interspecific variation, but they differ in that our finding demonstrated a relatively stronger effect of area than of isolation. We qualify this finding by noting that our data come from a landscape that is dominated by forest. The distance between isolated patches and continuous forest ranges from 150 to 900 m (*16*). Further forest destruction is expected to result in additional species loss and larger effects of isolation (*7*, *17*).

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Fig. S1
Tables S1 to S4
References

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Distinct Populations of Primary and Secondary Effectors During RNAi in *C. elegans*

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RNA interference (RNAi) is a phylogenetically widespread gene-silencing process triggered by double-stranded RNA. In plants and *Caenorhabditis elegans*, two distinct populations of small RNAs have been proposed to participate in RNAi: "Primary siRNAs" (derived from DICER nuclease-mediated cleavage of the original trigger) and "secondary siRNAs" [additional small RNAs whose synthesis requires an RNA-directed RNA polymerase (RdRP)]. Analyzing small RNAs associated with ongoing RNAi in *C. elegans*, we found that secondary siRNAs constitute the vast majority. The bulk of secondary siRNAs exhibited structure and sequence indicative of a biosynthetic mode whereby each molecule derives from an independent de novo initiation by RdRP. Analysis of endogenous small RNAs indicated that a fraction derive from a biosynthetic mechanism that is similar to that of secondary siRNAs formed during RNAi, suggesting that small antisense transcripts derived from cellular messenger RNAs by RdRP activity may have key roles in cellular regulation.

Double-stranded RNA (dsRNA)–triggered gene silencing in eukaryotes appears universally to involve 21- to 25-nucleotide (nt) siRNA effectors. In *Drosophila* and mammals, siRNAs derive primarily from processing of longer duplexes by DICER nuclease, forming 21- to 25-nt duplexes possessing 5′-monophosphates, 3′-hydroxyl groups, and 2-nt 3′ overhangs (*1*, *2*). Along with this "primary" siRNA response, amplification of the RNA trigger population has been proposed to contribute to potency and persistence of gene silencing in several systems (*3*, *4*). Amplification mechanisms are accompanied in some cases by "transitive RNAi" phenomena in which dsRNA matching one mRNA region can silence targets bearing homology to other parts of the mRNA (*5*–*9*). Unlike the situation in plants where "spreading" of the effector population occurs bidirectionally relative to the target mRNA (*6*, *8*), transitive RNAi in *Caenorhabditis elegans* exhibits a strong bias toward sequences upstream of trigger homology (*7*, *9*). Transitive RNAi requires function of a putative RdRP (RRF-1 in *C. elegans* soma, SDE1/SGS2 in *Arabidopsis thaliana*), suggesting several conceivable means for secondary siRNA production (*6*, *7*). One possibility is that antisense primary siRNAs could act as primers in the RdRP-mediated synthesis of new dsRNAs on an mRNA template. Alternatively, primary siRNAs may merely guide the RdRP to a target, allowing unprimed synthesis (*10*, *11*) either at the cleaved end of the targeted transcript, at a location close to the trigger-target complex, or at a structure such as a free end that might be revealed as aberrant through consequences of the initial RNA-induced silencing complex (RISC)::target interaction.

To better understand signal amplification in *C. elegans*, we characterized small RNAs from animals undergoing RNAi against an abundantly expressed endogenous gene, *sel-1* (*12*). After reverse transcription, 245,420 18- to 25-nt RNAs were sequenced by means of single-molecule pyrosequencing (*13*). Among these sequences, 534 exhibited either a perfect match (428 instances) or single mismatches (106 instances) to *sel-1* mRNA (Fig. 1, A and B). A similar analysis of ~850,000 clones from animals not exposed to dsRNA yielded just one *sel-1* small RNA (*14*). Most *sel-1* small RNAs induced during interference (483) had an antisense orientation, consistent with previous hybridization-based analyses (*7*). Of the 51 sense strand clones, 22 showed complementarity to at least one antisense clone.

We observed an incomplete bias in siRNA positions relative to the trigger; of 138 antisense siRNAs outside the original trigger, 110 (80%) occurred on the 5′ side. This bias could certainly account for preferential detection of upstream secondary responses in functional and biochemical assays (*7*, *9*). Twenty-eight observed instances of small antisense RNAs completely downstream of the trigger homology were of particular interest, as these would not have been expected if the sole mode of amplification involved extension by RdRP of existing siRNA triggers that hybridize to the target transcript.

Exon-exon junctions offer a unique opportunity to unequivocally distinguish de novo synthesis of antisense nucleic acids from an mRNA template (*15*, *16*). We found 50 *sel-1* small antisense RNA sequences that span exon/exon junctions. Of these, 43 fall within the trigger [458 base pairs (bp) of *sel-1* cDNA sequence] and thus could have derived directly from triggering dsRNA. Six antisense exon-exon junction sequences upstream of the trigger were recovered (four matching perfectly and two with single mismatches). These imply de novo copying of the mature mRNA template (*16*).

The apparent scarcity of *sel-1* siRNAs suggested that the procedure for cloning small RNAs (including ligation of linkers to 3′ and 5′ ends) might underrepresent the siRNA population (*12*). To analyze small RNA termini in detail, we used a number of structure-specific treatments. Treatment of RNA with periodate followed by β elimination results in a shift on a denaturing acrylamide gel, indicating at least one unmodified (cis-diol) 3′ terminus (*17*). Ribonuclease T (RNaseT) requires a 3′-hydroxyl to degrade single-stranded RNA. Finally, Terminator exonuclease preferentially degrades substrates with a single 5′-phosphate. Although *sel-1* siRNAs are susceptible to both β elimination and RNaseT reactions, they are resistant to Terminator (Fig. 2A). Control synthetic 25-nt *sel-1* RNA oligonucleotides s with 5′-monophosphate and 3′-OH were sensitive to all three treatments. We surmised that *sel-1* siRNAs are blocked at their 5′ ends.

We next asked if we could design a cloning protocol that would not be biased by the structure at the 5′ end on an siRNA. The resulting protocol (fig. S3) avoids both (i) the requirement for ligation of the 5′ end of the RNA and (ii) the possibility that modified 5′ ends on small RNAs could affect enzymatic treatments of the paired cDNA strand. We detected 127 *sel-1* antisense sequences and zero sense sequences from 1612

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total clones using this protocol (Fig. 1C). For *sel-1* antisense sequences, this represents a 40-fold enrichment compared to the 5′-ligation-dependent cloning method, providing further evidence for a prominent population of 5′-blocked siRNAs.

Secondary siRNAs are still recovered in 5′-ligation-dependent cloning, albeit inefficiently, as indicated by the representation of sequences outside the trigger (presumably most siRNAs within the trigger are also secondary). Notably, we found that small antisense segments cloned with a 5′-ligation-independent procedure were on average 1 nt longer than those cloned with a 5′-ligation-dependent procedure (Fig. 2B; figs. S4 and S5). The substantial increase in incidence of *sel-1* clones that followed 5′-ligation-independent cloning indicates that the vast majority of small *sel-1* RNAs are modified on their 5′ ends, while at most 2 to 3% (SOM Text) have simple 5′-phosphate termini that are exposed in vivo or produced by 5′ cleavage during the cloning procedure. An assumption that sense and antisense are roughly equal in the primary siRNA pool leads to primary siRNA estimates of <0.6% of the total *sel-1* siRNA population and <0.05% of the total 21- to 25-nt RNAs in the animal.

The two methods of cloning were selective for different classes of endogenous small RNAs (table S1) (*18*, *19*). microRNAs (miRNAs) appeared much less frequently with the 5′-ligation-independent cloning method, seemingly replaced by endogenous small RNAs corresponding to antisense sequence from coding regions. This analysis suggests that miRNAs and small antisense RNAs could be comparably abundant in *C. elegans*, with 5′ modification of the small antisense RNAs accounting for the predominance of miRNA clones in libraries derived using ligation-dependent schemes. We observed 612 out of 245,420 clones from the 5′-ligation-dependent method and 9 out of 1612 clones from the 5′-ligation-independent method that were perfect antisense copies of exon/exon junctions, suggesting synthesis by RdRP acting on an mRNA template (fig. S6).

To further characterize the modification of siRNAs in *C. elegans*, we used a ligation assay and Terminator 5′-exonuclease treatment (both requiring a 5′-phosphate). Sensitivity of the predominant fraction of the siRNAs could be restored by sequential treatment with alkaline phosphatase (which removes any number of 5′-phosphates) and T4 polynucleotide kinase (which adds a single 5′-phosphate), suggesting that the 5′ modification was likely to involve additional 5′-phosphate groups on the siRNA (Fig. 3, A and B). How many phosphates do these molecules have on their 5′ ends? Examining relative gel mobilities of the native and dephosphorylated siRNAs, using a variety of gel porosities (and using a series of synthetic RNA markers with different numbers of phosphates), indicated that the predominant fraction

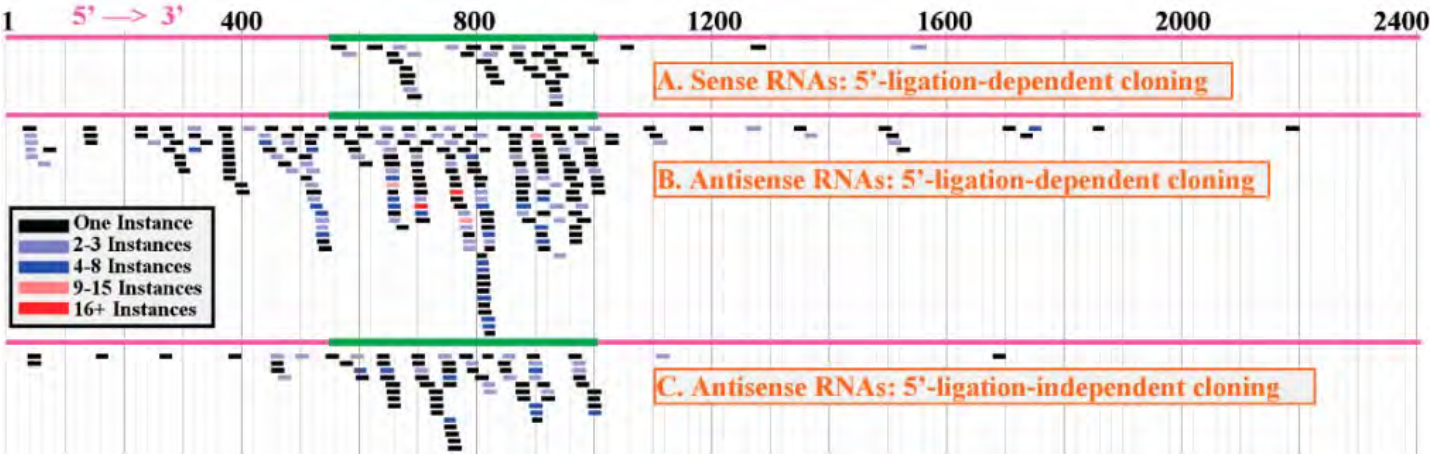

Fig. 1. Distinct RNAi-associated small RNA populations identified by 5′-ligation-dependent and 5′-ligation-independent methods. Small RNAs were isolated from animals in which RNAi had been triggered by dsRNA covering nucleotides 535 to 992 of *sel-1* mRNA. (**A** and **B**) By the 5′-ligation-dependent method, 51 sense clones (47 within the trigger, 4 downstream) and 483 antisense clones (110 upstream of the trigger, 1 in the trigger/upstream junction, 335 within the trigger, 9 in the trigger/downstream junction, 28 downstream) were obtained. (**C**) The 127 *sel-1*–associated small *RNAs* isolated by the 5′-ligation-independent method. All were antisense: 12 upstream of the trigger, 1 at the upstream/trigger junction, 104 within the trigger, and 3 downstream.

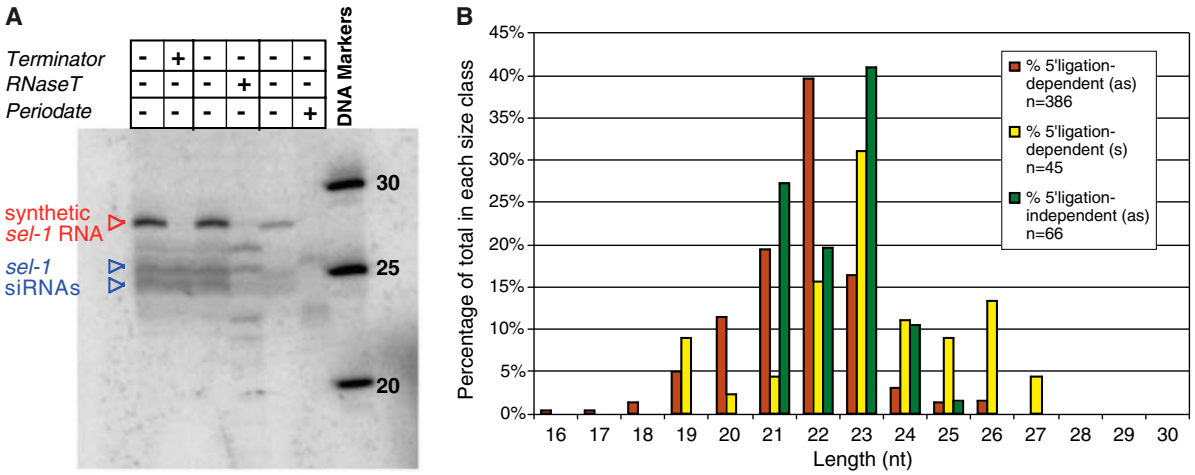

Fig. 2. Small RNAs associated with RNAi are modified at their 5′ termini. (**A**) *sel-1* small RNAs are protected from 5′-exonucleolytic attack but not from 3′ attack. Small RNAs were extracted from wild-type animals undergoing RNAi for *sel-1*; treated with the 5′-exonuclease Terminator, the 3′-exonuclease RNaseT, or sodium periodate plus β elimination; and subjected to Northern analysis (20% 19:1 acrylamide:bis-acrylamide gel) with probe directed against the *sel-1* trigger sequence (nt 535 to 992) (blue arrowheads). A control 25-nt synthetic *sel-1* RNA (5′-monophosphate) was added to each reaction (red arrowhead). (**B**) Size distributions of various classes of cloned small RNAs with perfect matches to *sel-1* sequence.

of the untreated siRNAs have triphosphate 5′ termini (Fig. 3, C and D).

The results presented here define an RNA population produced de novo during RNAi in *C. elegans* as a pool of 5′-triphosphate–terminated small antisense molecules templated by the mature mRNA target and covering sequences both upstream and downstream of the original dsRNA trigger. On the basis of this work and previous literature, our current working model for amplified gene silencing in *C. elegans* is that rare primary siRNAs, formed from a long dsRNA trigger, act as guides (presumably in an Argonaute-dependent manner) to recruit RdRP to targeted transcripts. This recruitment leads to de novo synthesis of short antisense RNAs that must be stripped off the template mRNA and incorporated into complexes that are capable of finding additional silencing targets. This model differs from other examples of RdRP action, such as in the generation of ta-siRNAs in *A. thaliana* where repeated DICER activity on long RdRP-generated dsRNAs produces a phased distribution of small RNAs (*20*).

Although ongoing RNAi is certainly important for de novo synthesis of antisense siRNAs, this process appears to contribute by providing guidance to the RdRP rather than priming activity. Primary silencing targets may or may not be degraded; whatever their fate, however, they remain intact for a sufficient period to be substrates for RdRP activity upstream and (somewhat less efficiently) downstream of the targeting site. This model is consistent with the biochemical properties of characterized cellular RdRPs in that these enzymes are capable of unprimed (as well as primed) synthesis (*10*, *11*). Initiation at 3′ ends of potential templates has been reported for fungal and plant RdRPs; this might allow initial Argonaute-mediated cleavage of mRNA targets to yield ready RdRP substrates.

Previous investigations of siRNA structure revealing double-stranded character, 3′ overhangs, and 5′-monophosphate termini were performed in organisms whose genomes do not encode canonical RdRPs (*2*, *21*, *22*). In addition, crystal structures of Argonaute proteins [key executioners in the RNAi pathway (*23*–*25*)] indicate considerable specificity in recognizing specific 5′ structures in RNA. It is possible that 5′-triphosphate antisense RNAs are themselves inactive in gene silencing, requiring either removal of two terminal phosphates or of the entire first base for activity. Alternatively, triphosphate-terminated small RNAs may be active directly as silencing triggers, potentially through distinct members of the Argonaute family that might recognize guide RNAs with a 5′-triphosphate.

One feature of the proposed mechanism is the involvement of dsRNA trigger only at the earliest stage of the process (production of primary siRNAs). Following this stage, the double-stranded character of the original trigger plays no role in the reaction. Given diverse structural features that could target an aberrant mRNA for RdRP activity, such a system would permit analogous machineries (each involving RdRP, helicase, and Argonaute activities) to serve in amplified surveillance processes triggered by both aberrant mRNA structure (*3*) and dsRNA (*26*).

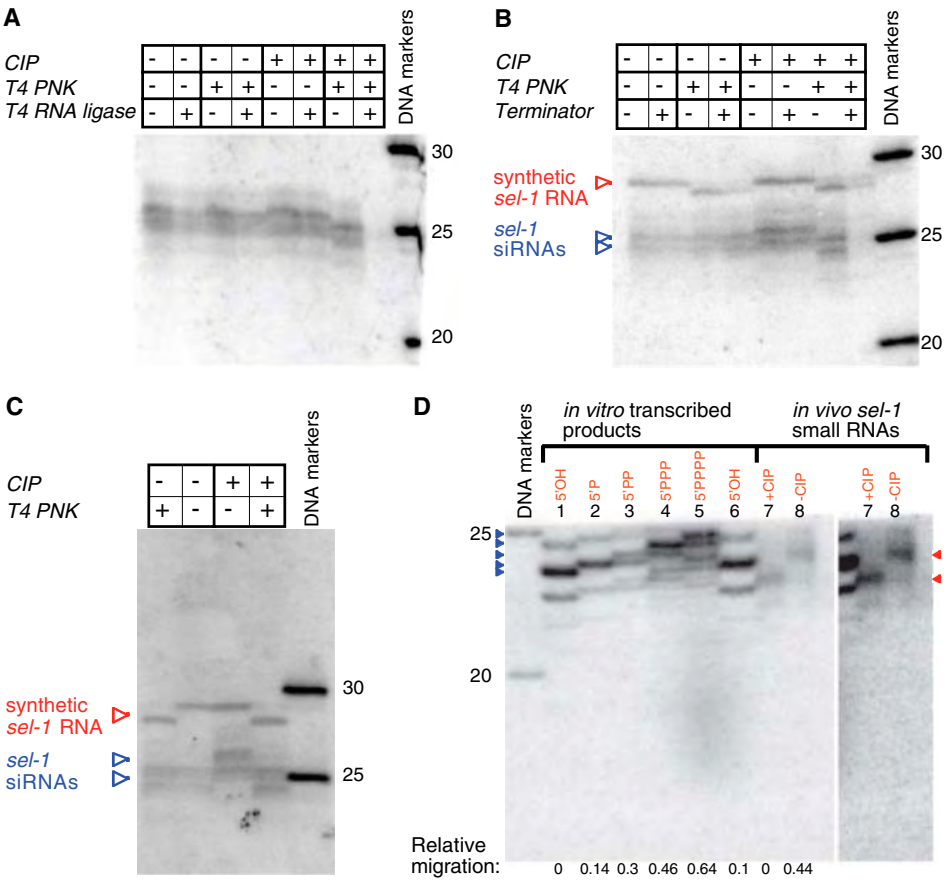

Fig. 3. Small RNAs associated with RNAi exhibit 5′-triphosphate termini. (**A**) Ligation assay shows that *sel-1* small RNAs possess multiple 5′-phosphates. Samples were sequentially treated with calf intestinal alkaline phosphatase (CIP), T4 polynucleotide kinase (T4 PNK), and/or ligase and analyzed by Northern blotting. Efficient ligation [leading to disappearance of the major siRNA bands (*27*)] requires 3′-OH/5′-monophosphate termini and was only observed after sequential treatment of siRNA populations with CIP (removing any number of 5′-phosphates) and T4 PNK (restoring a single 5′-phosphate). (**B**) Sensitivity to Terminator exonuclease, which preferentially degrades RNA with 5′-monophosphate termini. A 25-nt synthetic *sel-1* control RNA with 5′-OH terminus was included as an internal control (red arrowhead). Gels (A) and (B) used a 19:1 acrylamide:bis-acrylamide composition under which phosphatase-treated siRNAs comigrate with untreated siRNAs. These species are resolved on 75:1 acrylamide:bis gels (**C**), where untreated species migrate more quickly than 5′-OH RNAs. (**D**) *sel-1* small RNAs possess 5′-triphosphate termini. Control in vitro–synthesized RNAs with 5′-OH (lanes 1 and 6), 5′-monophosphate (lane 2), 5′-diphosphate (lane 3), 5′-triphosphate (lane 4), and 5′-tetraphosphate (lane 5) termini serve as mobility standards (blue arrowheads). With a high bis-acrylamide ratio (9:1 acrylamide:bis 12%), the size contribution of each 5′-terminal phosphate confers a prominent change in mobility such that a "ladder" of differentially 5′-phosphorylated species is observed. In vivo *sel-1* siRNAs were treated with CIP (lane 7) or mock-treated (lane 8) with a darker exposure of these lanes in the right panel (red arrowheads). The distance migrated through the gel relative to the 5′-OH–terminated species (lanes 2 to 6 were compared to lane 1 and lane 8 was compared to lane 7), given by the numbers in arbitrary units below each lane, demonstrated that in vivo siRNAs migrate as 5′-triphosphate–terminated RNAs.

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Materials and Methods
SOM Text
Figs. S1 to S6
Tables S1 and S2
References

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Secondary siRNAs Result from Unprimed RNA Synthesis and Form a Distinct Class

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In *Caenorhabditis elegans*, an effective RNA interference (RNAi) response requires the production of secondary short interfering RNAs (siRNAs) by RNA-directed RNA polymerases (RdRPs). We cloned secondary siRNAs from transgenic *C. elegans* lines expressing a single 22-nucleotide primary siRNA. Several secondary siRNAs start a few nucleotides downstream of the primary siRNA, indicating that non–RISC (RNA-induced silencing complex)–cleaved mRNAs are substrates for secondary siRNA production. In lines expressing primary siRNAs with single-nucleotide mismatches, secondary siRNAs do not carry the mismatch but contain the nucleotide complementary to the mRNA. We infer that RdRPs perform unprimed RNA synthesis. Secondary siRNAs are only of antisense polarity, carry 5′ di- or triphosphates, and are only in the minority associated with RDE-1, the RNAi-specific Argonaute protein. Therefore, secondary siRNAs represent a distinct class of small RNAs. Their biogenesis depends on RdRPs, and we propose that each secondary siRNA is an individual RdRP product.

RNAi is triggered by double-stranded RNA (dsRNA) molecules that are cleaved into 20- to 25-nucleotide (nt) siRNAs, which guide cleavage of homologous RNA molecules (*1*). Amplification of RNAi is inferred from the spreading of RNA silencing to regions outside the inducer sequence (transitive RNAi) (*2*–*4*) and involves the production of secondary siRNAs for which RdRPs have been implicated in nematodes, plants, and fungi (*5*). In vitro, RdRPs can perform both primed and unprimed RNA synthesis (*6*, *7*). In plants, the predominant route appears to be unprimed (5′ to 3′) RNA synthesis starting at the 3′ end of targeted transcripts and secondary siRNA production by DICER cleavages (*4*, *8*). Endogenous secondary siRNAs (trans-acting siRNAs, produced after miRNA-mediated mRNA cleavage) occur in a 21-nt register and correspond to both the sense and the antisense strand (*9*–*12*). Secondary siRNAs in *C. elegans* differ from those in plants in two aspects: The secondary siRNAs are only found upstream of the initial dsRNA trigger (*3*, *13*), and they are only of antisense polarity (*14*, *3*). Whereas trigger-derived primary siRNAs are bound by RDE-1, the secondary siRNAs are bound by a different set of Argonaute proteins (*15*). It is unknown how secondary siRNA production in *C. elegans* proceeds—whether RdRP-dependent cRNA synthesis starts primed on a primary siRNA or unprimed at the primary target site. Although the size of these secondary siRNAs (20 to 25 nt) (*3*) suggests that they are DICER products, their single polarity is difficult to explain.

To obtain a single and well-defined entry point of transitive RNAi, we constitutively expressed a single 22-nt primary siRNA against the *unc-22* body wall muscle gene from a miRNA-like transgene (fig. S1A). Worms transgenic for this 22si-gene show a twitching phenotype indicative of *unc-22* silencing, and *unc-22* mRNA expression is reduced by 50% (fig. S1B). Transitive RNAi in 22si lines has the characteristic *C. elegans* pattern, because secondary siRNAs occur only upstream of the primary target site and are only of antisense polarity (Fig. 1A). The 22si twitching phenotype is dependent on *rde* (RNAi-deficient) genes, such as *rde-1*, and not on miRNA-implicated genes, such as *alg-1* or *alg*-2 (Table 1). Apparently, the 22siRNA functions as an siRNA, even though it is processed from a primary miRNA-like transcript (fig. S1A), and accordingly (*16*), 22siRNA-mediated RNAi depends on matching of nucleotides 10, 11, and 12 to the target (fig. S1, A and C to E). Secondary siRNAs are absent in mutant backgrounds that result in nonphenotypic nematodes (Table 1), confirming that RNAi amplification is required for a 22si twitching phenotype. The primary 22siRNA was detected in all mutant backgrounds tested (Table 1), including *rde-1* and *rde-4* that were previously shown to be deficient in producing primary siRNAs (*17*, *18*). Size fractionation experiments revealed that in a wild-type (WT) background, the 22siRNA occurs in a complex of 100 to 150 kD that represents 22siRNA bound to RDE-1 (Fig. 1B), which is presumably the active silencing complex. miRNA complexes are larger (250 to 500 kD) (Fig. 1B) (*19*). In *rde-1* and *rde-4* mutants, the 22siRNA occurs in a 500-to 550-kD complex and cofractionates with DCR-1 (Fig. 1B). The passenger strand (the strand of an siRNA-duplex not incorporated into RISC), which is below detection in a WT background, accumulates in *rde-1* and *rde-4* mutants and cofractionates with the guide strand (the RISC-incorporated siRNA strand) (Fig. 1B). We infer that, in the absence of RDE-1 or RDE-4, the 22siRNA duplex remains in the DICER complex and the active silencing complex fails to be generated.

To determine the repertoire of secondary siRNAs generated during 22siRNA silencing, we cloned small RNAs by using a linker-independent protocol and sequence-selected secondary siRNAs by using biotinylated *unc-22*–specific oligonucleotides. Two different biotin oligonucleotides were used; oligo 1, which selects for secondary siRNAs in the 75-nt region immediately upstream of the primary siRNA (Fig. 2A); and oligo 2, which selects for primary and secondary siRNAs in a 50-nt region around the 22si target site (Fig. 2B). Consistent with ribonuclease protection assay (RPA) analyses (Fig. 1A), the cloned secondary siRNAs are of antisense polarity only and have a predominant length of 21 to 22 nt (table S1). Their production starts a few nucleotides downstream of the primary siRNA target site (Fig. 2B; table S2, column 1), which shows that non–RISC-cleaved mRNAs are among the substrates for secondary siRNA production. Because no further downstream secondary siRNAs are detected in RPA analyses (using 500-nt probes) (Fig. 1A), we infer that RdRP recruitment is restricted to the region around the primary siRNA. The generation of secondary siRNAs is a regulated process because similar sets of secondary siRNAs were cloned (Fig. 2A; table S1) in independent 22si worm lines and cloning experiments. RNA gel blots detecting secondary siRNAs showed that most of the 22si

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signal is RdRP-independent and represents primary siRNAs (fig. S2A). The secondary siRNAs appear to be in a phased register, suggesting that secondary siRNA production occurs via consecutive steps: either by progressive cleavages on a cRNA (by, e.g., DICER) or by multiple rounds of amplification (secondary siRNAs invoking tertiary siRNA production).

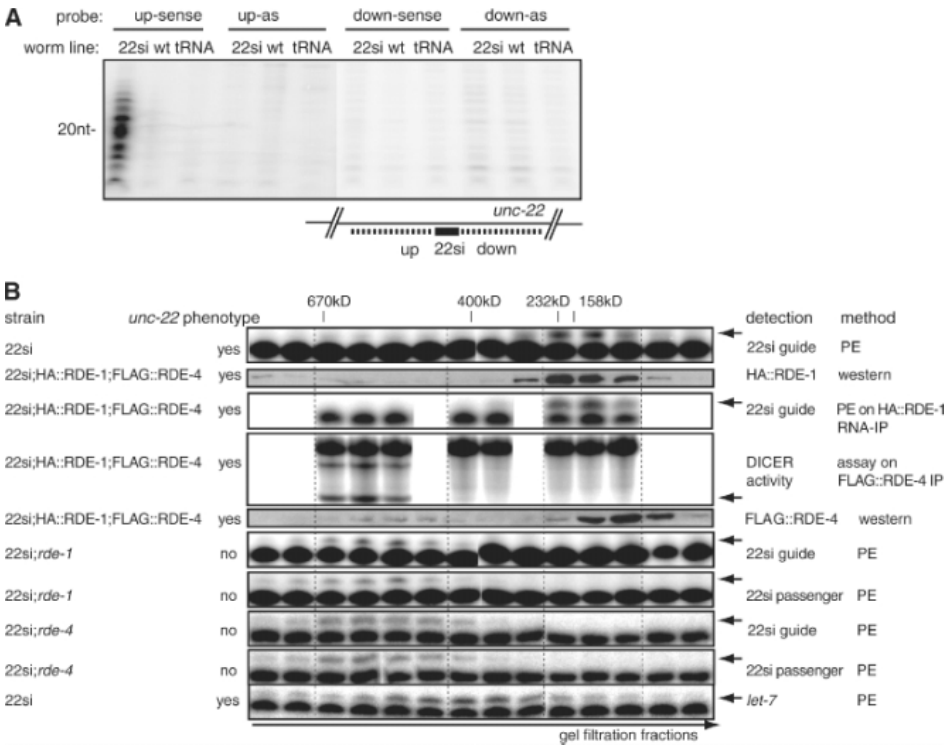

Fig. 1. Expression of a 22-nt *unc-22* siRNA from miRNA-like transgene results in *unc-22* transitive RNAi that is dependent on *rde-1, rde-4*. (**A**) Secondary siRNAs in 22si transgenic lines occur only for the region upstream of the primary siRNA and are only of antisense polarity, as analyzed by RPAs with 500-nt single-stranded probes. (**B**) Analyses of size fractions. Immunoprecipitation (IP), RNA-IP, primer extension (PE) with 18-nt end-labeled oligonucleotides, Western blot analysis, and DICER activity assay were used to determine protein and RNA composition in the various gel filtration fractions. Arrows indicate the 22-nt products generated by primer extension or DICER activity. Empty spaces indicate that the size fraction was not tested.

DICER products have 5′-monophosphates and 2′,3′-hydroxy termini. miR-58 and 22si (which are both cleaved by DICER from an miRNA precursor) and the secondary siRNAs carry terminal 2′,3′-hydroxyl groups, because $NaIO_4$-reacted, β-eliminated RNAs (*20*) migrate ~1 nt faster than the untreated RNAs (Fig. 2C and fig. S2B). Like miR-58 and 22siRNA, the secondary siRNAs contain 5′-phosphates, because alkaline phosphatase–treated RNAs migrate about half a nucleotide slower than untreated RNAs (Fig. 2C and fig. S2B). In contrast to miR-58 and 22si molecules, untreated secondary siRNAs migrate somewhat slower than their 5′-monophosphorylated derivatives (Fig. 2C and fig. S2B), suggesting that secondary siRNAs carry a different number of 5′-phosphates. Absence of a 5′-monophosphate was also inferred from ligation experiments with T4 RNA ligase, which requires a 5′-monophosphate; the 5′ linker successfully ligated to miR-58 and 22siRNA but failed to ligate to secondary siRNAs (Fig. 2D). Secondary siRNAs have 5′ di- or triphosphate because they can be capped with vaccinia virus capping enzyme, as deduced from a 2-nt slower migration (Fig. 2E). A minority of 22siRNAs can be capped and represent secondary siRNAs of the same sequence as the primary siRNA (Fig. 2E). From these findings we infer that it is unlikely that secondary siRNAs in *C. elegans* are products of consecutive DICER cleavages, although we cannot exclude that they are modified after dicing. Alternatively, they either are 21- or 22-nt cRNAs (short RdRP products), or they are cleaved from longer cRNAs by an alternative nuclease or by an alternative way of dicing.

Several alternatives exist for initiation of secondary siRNA production: (i) cRNA synthesis occurs primed on 22siRNA molecules; (ii) cRNA synthesis starts unprimed at 22si-RISC–

Table 1. Genetic requirements for 22si function.

Background	Predicted/known function	*unc-22* phenotype	Secondary siRNAs*	22si siRNA†	22si in size fractionation‡
WT	WT	Yes	Yes	Yes	RDE-1 peak
dcr-1	dsRNase miRNAs + siRNAs	Yes§‖	ND¶	ND	ND
alg-1	Argonaute miRNA	Yes	ND	Yes	RDE-1 peak
alg-2	Argonaute miRNA	Yes	ND	Yes	RDE-1 peak
tsn-1	RISC component *Drosophila*	Yes	Yes	Yes	RDE-1 peak
vig-1	RISC component *Drosophila*	Yes	ND	ND	ND
rde-1	Argonaute siRNAs	No	No	Yes	DICER peak
rde-4	RNAi (DICER cofactor)	No	ND	Yes	DICER peak
rde-3	RNAi (nucleotidyltransferase)	No	No	Yes	RDE-1 peak
rrf-1	Somatic RNAi (RdRP)	No	No	Yes	RDE-1 peak
rde-2	Germline RNAi	Yes	ND	Yes	RDE-1 peak
mut-15	RNAi	No	No	Yes	RDE-1 peak
mut-16	Germline RNAi	No	No	Yes	RDE-1 peak
rrf-3	Suppressor RNAi (RdRP)	Severe#	Yes	Yes	RDE-1 peak
eri-1	Suppressor RNAi (siRNase)	Severe	Yes	Yes	RDE-1 peak
rrf-1;eri-1	RNAi + suppressor RNAi	No	ND	Yes	RDE-1 peak
rrf-1;rrf-3	RNAi + suppressor RNAi	No	ND	Yes	ND
rde-1;eri-1	RNAi + suppressor RNAi	No	ND	ND	ND
rde-1;HA::Rde-1;FLAG::Rde-4	RNAi + rescue	Yes	ND	Yes	RDE-1 peak

*Determined by RPAs. †Determined by primer extension analysis. ‡Representative patterns in Fig. 1D. §Maternal contribution may influence analysis. ‖Some nonphenotypic *unc-22* nematodes were observed when *dcr-1*–specific RNAi (feeding assay) was performed on 22si;*eri-1* worms. ¶Not determined. #Nematodes are paralyzed, which represents a severe *unc-22* phenotype compared to twitching.

cleaved mRNA; and (iii) RdRPs are recruited to an mRNA targeted by a 22siRNA, and unprimed cRNA synthesis occurs by internal entry around the target site. Occurrence of this third route is supported by the finding that secondary siRNA production occurs a few nucleotides downstream of the primary siRNA. To investigate whether cRNA is generated by elongation of the primary siRNA, we generated two new transgenic lines in which the 22siRNA carries a single mismatch to the *unc-22* target mRNA at position 21 or position 11. Replacement of the mismatches within the secondary siRNAs would show unprimed cRNA synthesis, whereas primed synthesis would result in maintenance of the mismatched nucleotide in the secondary siRNA. Both 22si-mm21 and 22si-mm11 transgenic nematodes show a weak twitching phenotype indicative of *unc-22* silencing and produce relatively low levels of secondary siRNAs (Fig. 3A), possibly due to the presence of a single-nucleotide mismatch. Sequence analysis of secondary siRNAs that have overlap with the primary siRNA showed that for all these secondary siRNAs, both mm21 and mm11 had been replaced by the nucleotide complementary to the mRNA (Fig. 3, B and C; table S2). Thus, secondary siRNA production

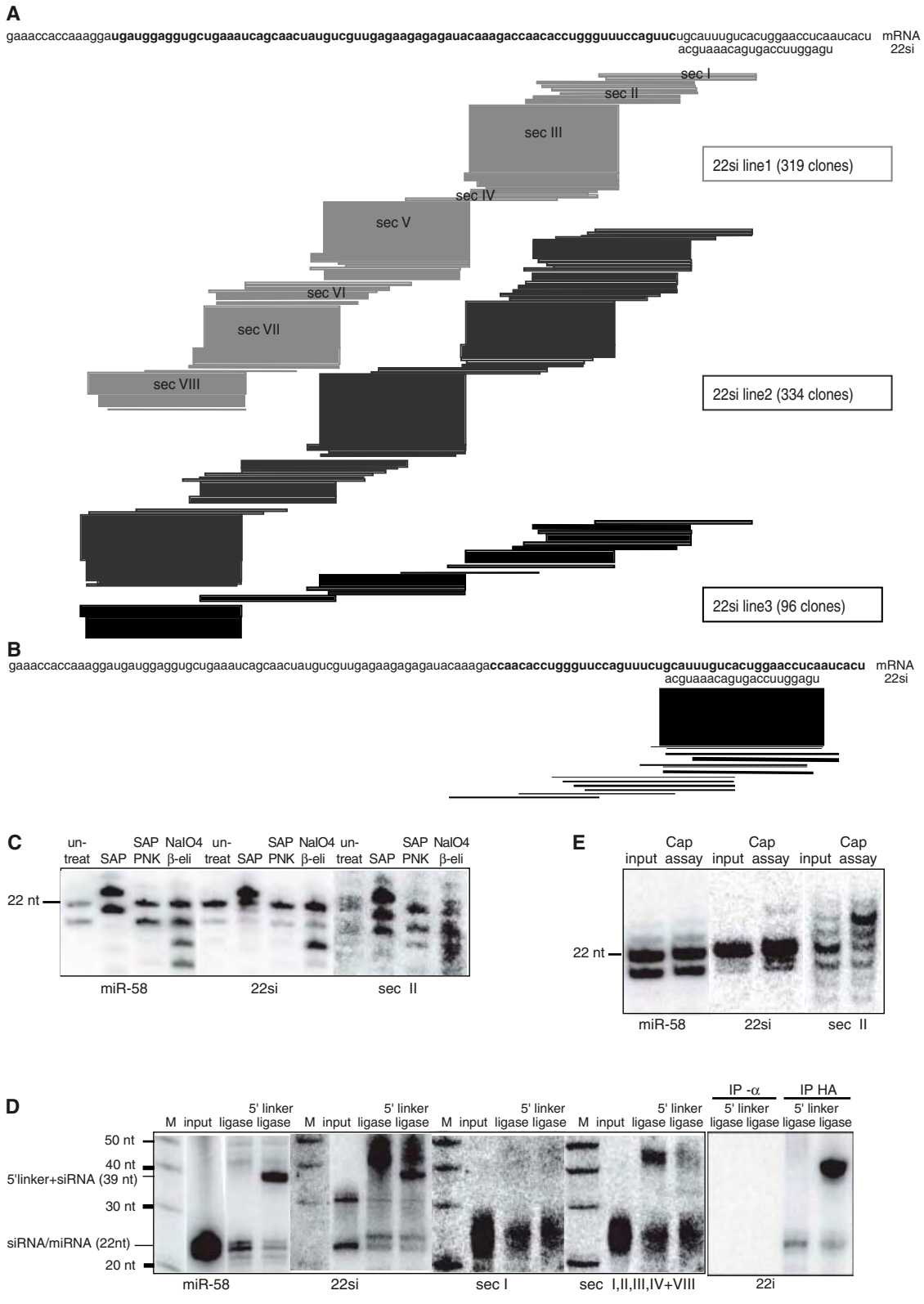

Fig. 2. Secondary siRNA sequences, frequencies, and properties. (**A**) Schematic representation of secondary siRNAs cloned from three independent 22si lines with biotinylated oligo 1 (bold sequence). Secondary siRNAs are of antisense polarity. The thickness of the lines representing the secondary siRNAs indicates the number of clones. (**B**) Secondary siRNAs cloned from 22si line 3 in a *eri-1* background with biotinylated oligo 2 (bold sequence). (**C**) Migration of untreated, dephosphorylated [shrimp alkaline phosphatase (SAP)–treated], monophosphorylated [SAP- and T4 polynucleotide kinase (PNK)–treated] and $NAIO_4$-reacted, β-eliminated RNAs. (**D**) RNA gel blot analysis of linker ligation assays with a 5′ linker and T4 RNA ligase. M, RNA marker. Input consists of gel-isolated 15- to 30-nt RNA fraction or RNAs isolated from IPs with a hemagglutin (HA)–specific antibody on 22si;HA::RDE-1;FLAG::RDE-4 nematodes. (**E**) RNA gel blot analysis of capping assay.

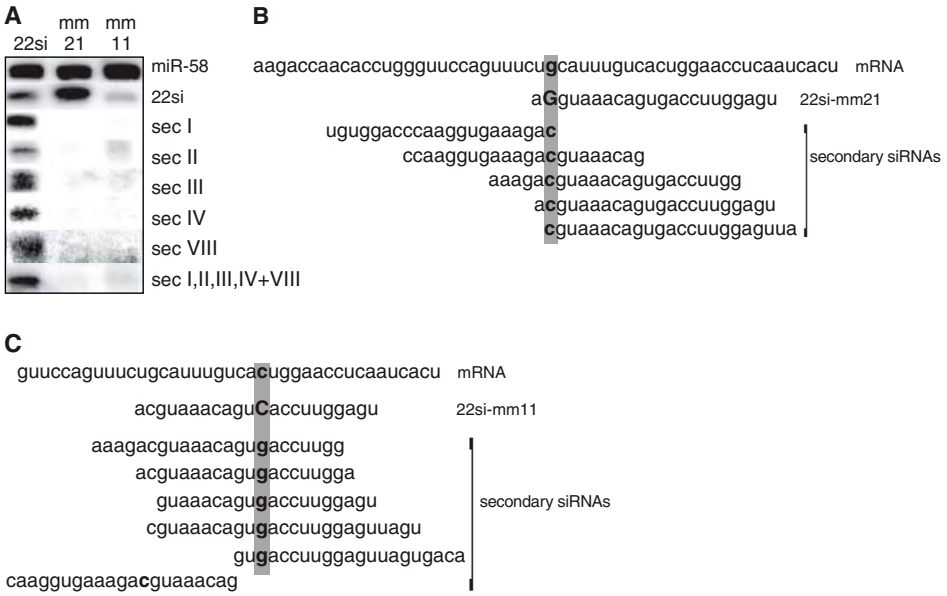

Fig. 3. Secondary siRNAs result from unprimed cRNA synthesis. (**A**) RNA gel blot detection of primary and secondary siRNAs in 22si- and 22si-mm10,11,12 lines. (**B**) Sequence of secondary siRNAs cloned from 22si-mm21 lines. The gray bar indicates mismatch 21. (**C**) Sequence of secondary siRNAs cloned from 22si-mm11 lines. The gray bar indicates mismatch 11.

occurs only unprimed. The 22siRNA is bound by RDE-1 (Fig. 1B and fig. S3A), which carries the DDH motif needed for RISC cleavage (*21*). The presence of a mismatch at the presumed RISC cleavage site in the 22si-mm11 primary siRNA did not alter the range of secondary siRNAs that were cloned (Fig. 3C, table S2), suggesting that nematode RNAi does not primarily depend on RISC cleavage of mRNAs. A minority of the secondary siRNAs is loaded into the RNAi-specific argonaute protein RDE-1 (fig. S3, A and B), and most secondary siRNAs may be loaded into secondary Argonaute proteins (*15*) (fig. S3C). The phased register in which the secondary siRNAs appear to be ordered (Fig. 2A) may arise from multiple amplification rounds (by RDE-1–loaded secondary siRNAs) or from preferred initiation sites of cRNA synthesis.

Secondary siRNAs represent a distinct class of 21- to 22-nt small RNAs that can can silence mRNAs in trans (*3*). They result from unprimed RNA synthesis (Fig. 3, B and C), are only of antisense polarity (Fig. 1A), carry 5′ di- or triphosphates (Fig. 2, C to E), and are mainly loaded into a distinct set of Argonaute proteins (*15*) (fig. S3). Secondary siRNAs can be produced on non–RISC-cleaved mRNAs presumably by internal entry of the RdRP. A 3′ nonprogressive DICER cleavage may be responsible for generating the secondary siRNA 3′ end and degrading the mRNA (fig. S4). We find that for the 22siRNA, a single-nucleotide mismatch decreases silencing efficiency considerably. Likely, these strict prerequisites for inducing transitive RNAi protect organisms against unintended off-target effects (*22*, *23*).

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Materials and Methods
Figs. S1 to S4
Tables S1 and S2
References

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Physiological Proteomics of the Uncultured Endosymbiont of *Riftia pachyptila*

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The bacterial endosymbiont of the deep-sea tube worm *Riftia pachyptila* has never been successfully cultivated outside its host. In the absence of cultivation data, we have taken a proteomic approach based on the metagenome sequence to study the metabolism of this peculiar microorganism in detail. As one result, we found that three major sulfide oxidation proteins constitute ~12% of the total cytosolic proteome, which highlights the essential role of these enzymes for the symbiont's energy metabolism. Unexpectedly, the symbiont uses the reductive tricarboxylic acid cycle in addition to the previously identified Calvin cycle for CO_2 fixation.

Riftia pachyptila inhabits deep-sea hydrothermal vent areas along mid-ocean ridges in the East Pacific (*1*). Instead of containing a digestive system, the worm's coelomic cavity is densely populated by a single species of sulfide-oxidizing gamma-proteobacteria that provide for their host's carbon and energy supply by fixing CO_2 from the surrounding water (*2*–*4*). Microbial chemosynthesis is sustained by the presence of H_2S originating from reduced hydrothermal fluids and oxygen in the seawater (*5*). Compared with free-living sulfur oxidizers, the symbionts benefit from high nutrient concentrations within the worm's body (*6*), which allow for a high metabolic activity. The microbially produced carbon compounds are transferred to the host, making *R. pachyptila* one of the fastest-growing marine invertebrates known (*7*).

We took a functional genomics approach to analyze the proteome and hence to derive

information on the physiology of the uncultured *Riftia* symbionts. The sequencing of the symbiont genome was conducted by a metagenome approach. Bacterial symbionts were isolated and separated from the host tissue, the so-called trophosome (*8*). Using one- and two-dimensional (2D) gel electrophoresis, we established reference maps (master gels) of the soluble intracellular and the membrane-associated bacterial proteome, on which more than 220 identified proteins have been registered so far (Fig. 1 and fig. S1). Our procedures were reproducible and showed the presence of a single endosymbiont species (*9*) with no indication of other bacterial or host tissue contaminants. The proteomic approach provided evidence regarding which of the predicted genes are expressed under natural growth conditions. Levels of protein synthesis reflect the relative proportion of translational capacity that is invested in the individual metabolic paths, and we can deduce the relevance of particular proteins for the symbionts' physiology. On the basis of our proteome data, we were thus able to deduce major pathways of the symbionts' metabolism, e.g., the sulfide oxidation pathway (fig. S2) and the reverse tricarboxylic acid (TCA) cycle (fig. S3), as well as to make inferences about the symbionts' response to oxidative stress.

Although great advances have been made in our understanding of the sulfur oxidation pathways in a variety of sulfur oxidizers in recent years, this pathway remains incompletely characterized in *Riftia* symbionts. In this proteome study, the enzymes DsrA (dissimilatory sulfite reductase), AprA/AprB (adenosine phosphosulfate reductase), and SopT [adenosine triphosphate (ATP) sulfurylase] were identified, which suggested the reactions displayed in fig. S2: H_2S is

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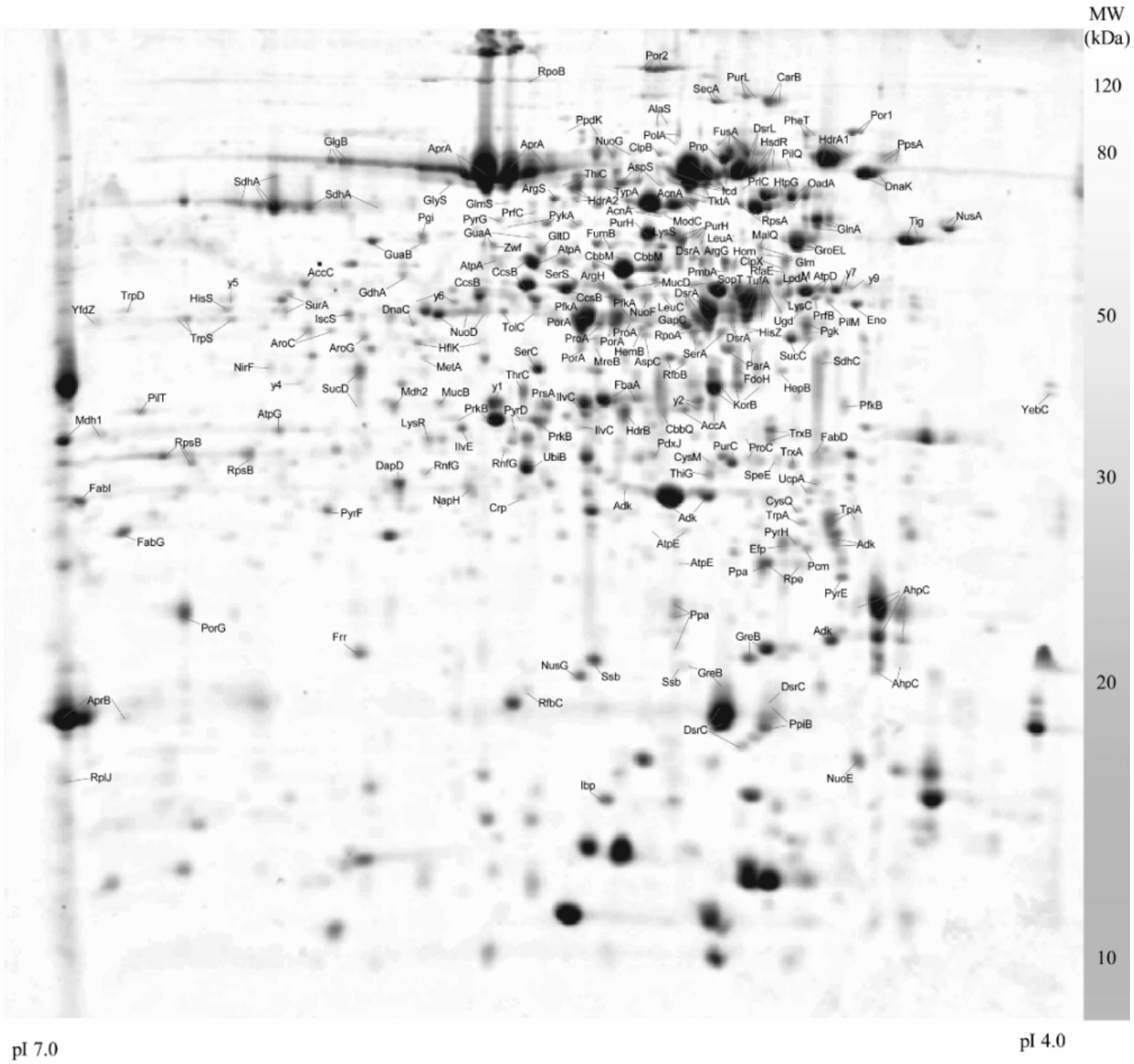

Fig. 1. Reference map of the *Riftia pachyptila* endosymbionts' intracellular proteome. Identified symbiont proteins are indicated. Protein functions are listed in table S1.

oxidized to SO_3^{2-} in a six-electron step; this is subsequently converted to adenosine phosphosulfate (APS), with consumption of adenosine monophosphate (AMP). The final oxidation step yields ATP by substrate-level phosphorylation and simultaneously creates the end product, SO_4^{2-}. Although these enzymes were originally described as part of the reverse pathway in sulfate-reducing bacteria (as reflected in their names), it has long been proposed that they can function just as well in the opposite direction (*10*). Quantitative analyses of the respective protein spots in our study revealed that the three major sulfide oxidation proteins DsrA, AprA/AprB, and SopT constitute more than 12% of the total cytosolic symbiont proteome in a pH range of 4 to 7. This highlights the essential role of these enzymes for the symbiont's energy metabolism.

Table 1. Comparison of *Riftia* symbiont protein spot intensities under high- and low-sulfur conditions. Spot intensities of selected proteins were compared in 2D gel images from sulfur-rich and sulfur-depleted trophosome samples (see SOM for details). Positive ratios represent the factor by which the respective spot intensity was higher on the gels from sulfur-rich trophosomes. Negative ratios give the factor by which spot intensity was higher under low-sulfur conditions. The listed protein spots are highlighted in Fig. 2.

Protein	Function	Spot ratio
	Proteins with higher spot intensity in sulfur-rich trophosome samples	
AprA	Adenylylsulfate reductase, α subunit	8.20
CbbM	RuBisCO form II, large subunit	4.29
DsrA	Dissimilatory sulfite reductase, α subunit	3.55
HdrA2	Heterodisulfide reductase, subunit A	2.26
DsrL	Predicted NADPH:acceptor oxidoreductase	1.92
DsrC	Dissimilatory sulfite reductase, γ subunit	1.47
AprB	Adenylylsulfate reductase, β subunit	1.30
	Proteins with higher spot intensity in sulfur-depleted trophosome samples	
SdhA	Putative fumarate reductase, flavoprotein subunit	−23.83
OadA	Oxaloacetate decarboxylase, α subunit	−15.00
Por1	Pyruvate:flavodoxin/ferredoxin oxidoreductase	−8.58
SucC	Succinyl CoA ligase, β subunit	−6.75
PorG	Pyruvate:ferredoxin/flavodoxin oxidoreductase, γ subunit	−6.13
KorB	2-Oxoglutarate synthase, β subunit	−4.57
AcnA	Aconitase A	−4.50
Mdh1	Malate dehydrogenase	−2.36
SucD	Succinyl-CoA synthetase, α subunit	−2.12
PorA	Pyruvate:flavodoxin/ferredoxin oxidoreductase, α subunit	−2.03

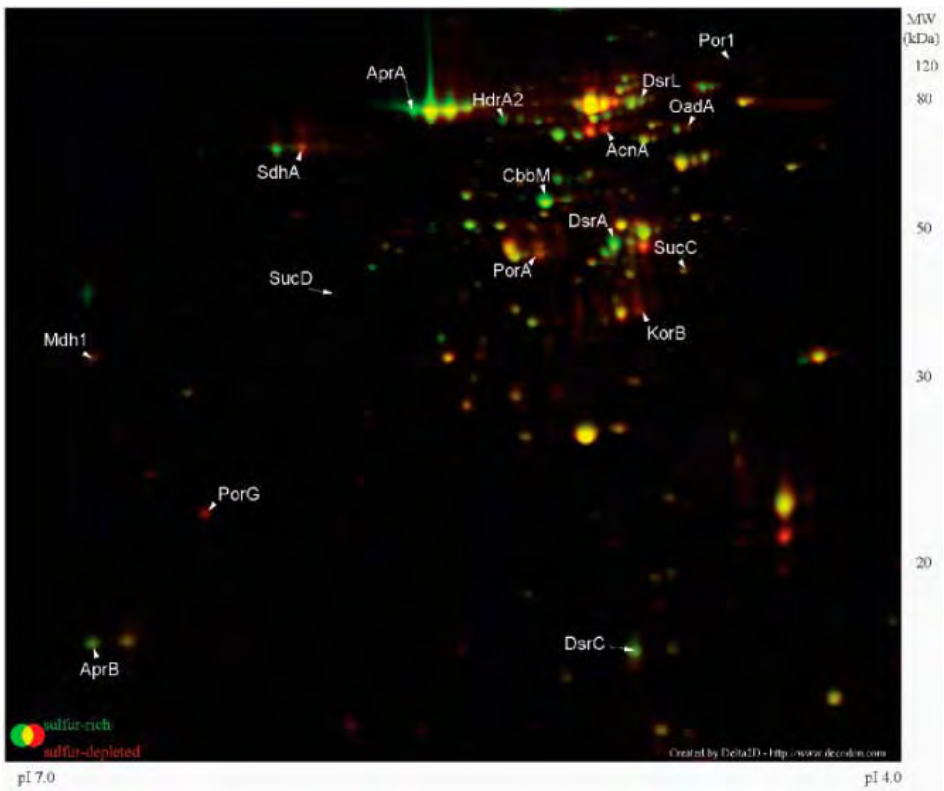

Fig. 2. Comparison of protein patterns under high- and low-sulfur conditions. Images of the 2D gels were colored (sulfur-rich, green, and sulfur-depleted tissue, red spots) and overlaid. Selected proteins with distinct variations in their relative spot volumes from one gel to the other are indicated (see SOM for details).

Using our intracellular protein master gel, we also examined the *Riftia* symbiont's carbon metabolism. We identified most of the enzymes involved in the energy-generating TCA cycle. Moreover, we found the enzyme 2-oxoglutarate: ferredoxin oxidoreductase β subunit (KorB), several copies of a pyruvate:ferredoxin oxidoreductase (subunits Por1, Por2, and PorA/PorG), a putative fumarate reductase (subunits SdhA/SdhC), and a protein highly similar to a citryl–coenzyme A (CoA) synthetase subunit (CcsB). These enzymes could run the TCA cycle in the reductive direction (fig. S3). This cycle represents an alternative CO_2 fixation mechanism that requires less energy than the Calvin cycle for each three-carbon unit formed. Considering the great abundance of these four key enzymes on the protein gels (see relative spot volumes in table S1), we suggest that the reductive TCA cycle is not only a possibility, but a very important feature of the *Riftia* symbiont's carbon metabolism.

To verify the symbiont's capability for using the reductive TCA cycle for CO_2 fixation, we tested cell extracts of isolated bacteria for activity of the above-mentioned key enzymes: The assays clearly revealed specific enzyme activities in all four cases that are higher than those previously reported for ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) (*11*) (table S2).

The results of our proteome analysis, in combination with the measured enzyme activities, provide strong evidence that the *Riftia* symbionts, which have been considered a prime example for chemolithoautotrophic carbon fixation via the Calvin cycle, use, at least partly, the reductive TCA cycle for autotrophic carbon fixation as well. This might explain the long-standing dilemma that the stable carbon isotopic composition of the *Riftia* symbiont is substantially heavier ($\Delta^{13}C$ of –9 to –16‰) than would be expected by the use of the Calvin cycle alone (*12*). The observed isotopic variation most likely results from varying contributions by the reductive TCA cycle and the Calvin cycle. It is also interesting to note that, concordant with previous findings (*12*), RuBisCO constitutes only ~1% of the *Riftia* symbiont's total protein on our gels. This is a small percentage compared with other bacteria that use only the Calvin cycle for CO_2 fixation, where RuBisCO is usually the major protein and can account for 4 to 50% of the total soluble protein (*13*). The apparent discrepancy between the low concentration and the relatively high activity of RuBisCO in the trophosome (*4*) and the possible occurrence of two carbon fixation pathways in one organism certainly warrant further investigations.

Riftia symbionts can store elemental sulfur in their periplasm if high concentrations of H_2S are available (*14*); these stores result in a light green, almost yellow trophosome. The tissue appears dark green or black (*15*) when sulfur is limiting. To analyze the symbionts' potential responses to changing environmental conditions, we compared bacterial protein patterns from naturally occurring sulfur-rich and from sulfur-depleted trophosome tissues. Under high-sulfide conditions, the resulting 2D gels revealed a distinctly higher spot intensity for enzymes involved in sulfide oxidation and for RuBisCO, compared with low-sulfur conditions (Table 1, Fig. 2). Spot intensities of the sulfide oxidation enzymes AprA and DsrA were about eight and four times, respectively, those under low-sulfur conditions. This indicates that *Riftia* symbionts are capable of adjusting the production of enzymes needed for energy metabolism to the prevailing environmental conditions. In bacteria from tissue with little or no stored sulfur (dark trophosomes), the protein spot volume of the putative fumarate reductase subunit SdhA was about 24 times as high as, and the spot volume of the putative pyruvate:ferredoxin oxidoreductase Por1 was about 9 times as high as that in sulfur-rich, light trophosome (Fig. 2, Table 1). Several other enzymes involved in the reductive TCA cycle, including two other pyruvate:ferredoxin oxidoreductase subunits, PorG and PorA, and the 2-oxoglutarate synthase subunit KorB, could also be detected with elevated spot volumes in samples from sulfur-depleted trophosomes. This suggests that the *Riftia* symbiont might be capable of adapting to a temporary low-energy situation by adjusting its way of carbon fixation: The use of the Calvin cycle might be reduced in favor of the up-regulation of the energetically more favorable reductive TCA cycle. Because *Riftia* symbionts have also been shown to produce glycogen as a carbon storage compound (*16*), it might even be speculated that—for example, under long-lasting or severe low-energy conditions—their metabolism switches from an autotrophic mode to a heterotrophic mode. In this case, the symbiont might revert to burning carbon reserves through glycolysis and the oxidative TCA cycle to generate energy and precursors for biosynthesis. The symbiont might thus be able to use the TCA cycle in the oxidative and reductive direction, depending on the environmental conditions, which would allow for a high metabolic flexibility. Apparently, the true nature of the *Riftia* symbiont's metabolic strategies is much more complex than expected. Further detailed investigations are needed to explicitly evaluate and validate these hypotheses.

Physiological tests revealed that *Riftia* symbionts do not have a catalase to protect the cells from H_2O_2 (*17*). This result is supported by the lack of the respective gene in the metagenome sequence. Because *Riftia* symbionts do not tolerate high oxygen concentrations (*18*), we tested their strategy of coping with stress situations caused by hydrogen peroxide. Our experiments revealed a strong induction of the alkyl hydroperoxide reductase AhpC (Fig. 3). This enzyme is present in large amounts in the cytoplasmic protein fraction (Fig. 1) and reduces organic hydroperoxides caused by H_2O_2. Our results indicate that AhpC plays a crucial role in the resistance of these microaerophilic bacteria to oxidative stress.

This study shows that a comparative proteomic view of the *Riftia* symbionts' cell physiology allows for a complex physiological description without their cultivation. It reaches beyond the mere prediction of putative metabolic functions as coded in the genome sequence.

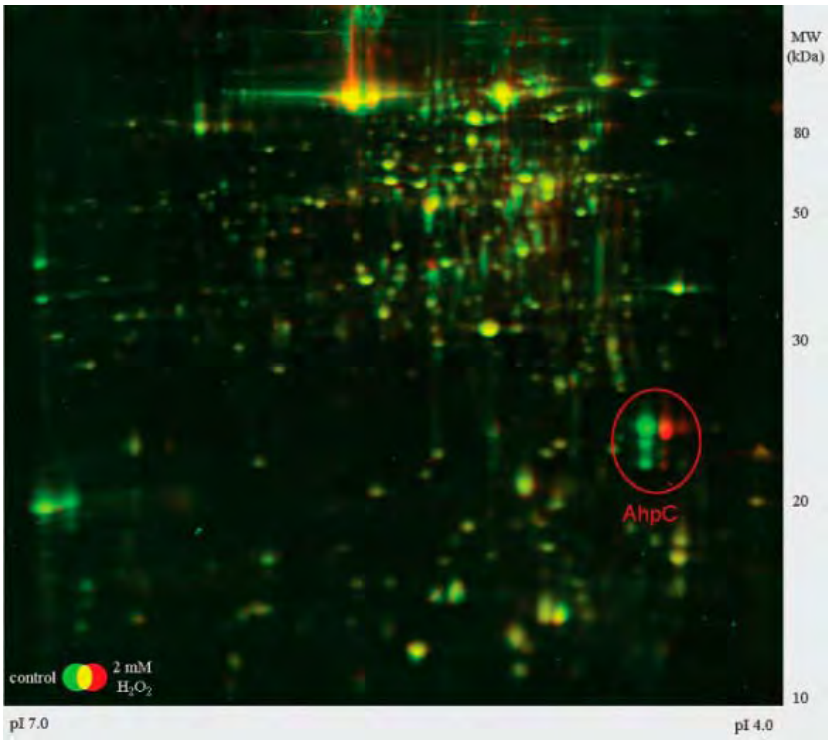

Fig. 3. Comparison of protein patterns in response to oxidative stress. The 2D gels show proteins from samples taken before and 60 min after the addition of 2 mM H_2O_2 to a suspension of isolated *Riftia* symbionts. Both images were colored (control, green spots, and stress sample, red spots) and overlaid. The oxidative stress protein AhpC is indicated. Because of the delayed deformylation of the newly synthesized protein during the massive induction of AhpC production, the protein spot shifts to a slightly more acidic pH. The "new" AhpC, produced after the stress with H_2O_2 is thus visible as red spots right next to the "old" AhpC, visible as green spots.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 and S2
References and Notes

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An H-NS–like Stealth Protein Aids Horizontal DNA Transmission in Bacteria

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The Sfh protein is encoded by self-transmissible plasmids involved in human typhoid and is closely related to the global regulator H-NS. We have found that Sfh provides a stealth function that allows the plasmids to be transmitted to new bacterial hosts with minimal effects on their fitness. Introducing the plasmid without the *sfh* gene imposes a mild H-NS$^-$ phenotype and a severe loss of fitness due to titration of the cellular pool of H-NS by the A+T-rich plasmid. This stealth strategy seems to be used widely to aid horizontal DNA transmission and has important implications for bacterial evolution.

Horizontal transfer of genetic material allows bacteria to acquire new traits, such as those contributing to virulence or symbiosis, or resistance to antibiotics and other antimicrobial agents (*1*, *2*). An unresolved issue in studies of horizontal transfer is how the newly acquired genes become embedded in the regulatory networks of the host in ways that promote the long-term interests of both the genes and the host (*3*). Importing even a structurally simple plasmid can impose a fitness cost on the bacterium (*4*–*6*), so one might anticipate that the arrival of large groups of genes could place the recipient at a competitive disadvantage.

R27 is a large plasmid that was isolated from *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) in the early 1960s (*7*) and has been detected more recently in *S.* Typhi outbreaks in India, Pakistan, and Southeast Asia (*8*, *9*). An IncHI1 plasmid called pSf-R27 that is 55% A+T and 99.7% identical to R27 was discovered in *Shigella flexneri* 2a strain 2457T (*3*, *10*–*12*). The plasmids differ in that pSf-R27 harbors no antibiotic resistance genes. Like other R27-like plasmids, pSf-R27 encodes a protein that is 59% identical to the DNA binding protein H-NS (*3*, *10*, *11*), an abundant nucleic acid binding protein found in many Gram-negative bacteria. H-NS binds to regions of curvature in A+T-rich DNA, allowing it to act globally as a repressor of transcription (*3*, *13*). The pSf-R27–encoded paralog, Sfh, can substitute fully for H-NS (and vice versa) in *Escherichia coli* and *S. flexneri* and has a similar preference for binding curved A+T-rich DNA sequences (*10*, *11*).

To test the hypothesis that horizontal transfer by conjugation of the *sfh* gene located on pSf-R27 could alter global gene expression patterns in transconjugants, we introduced plasmid pSf-R27, with or without a functioning *sfh* gene, into the mouse-virulent SL1344 strain of *S.* Typhimurium. We observed the effect on the transcriptome, competitive fitness, and virulence of the transconjugants. Surprisingly, the introduction of pSf-R27 resulted in relatively few changes in the recipient, whereas its Δ*sfh* derivative (pSf-R27Δ*sfh*) exerted many unexpected effects.

The native pSf-R27 plasmid altered the transcription of a limited number of *S.* Typhimurium genes (Fig. 1), with just 25 showing more than a 50% reduction (table S1) and 68 showing more than a doubling in transcription (table S2). In contrast, the introduction of the *sfh*-deleted plasmid pSf-R27Δ*sfh* resulted in altered expression of a much wider range of genes distributed across all categories (Fig. 1), with 119 genes down-regulated (table S3) and 323 genes up-regulated (table S4). Prominent among these were genes involved in bacterial virulence, motility, the DNA damage response, and central metabolism. The effect on genes contributing to each of these processes was confirmed by the

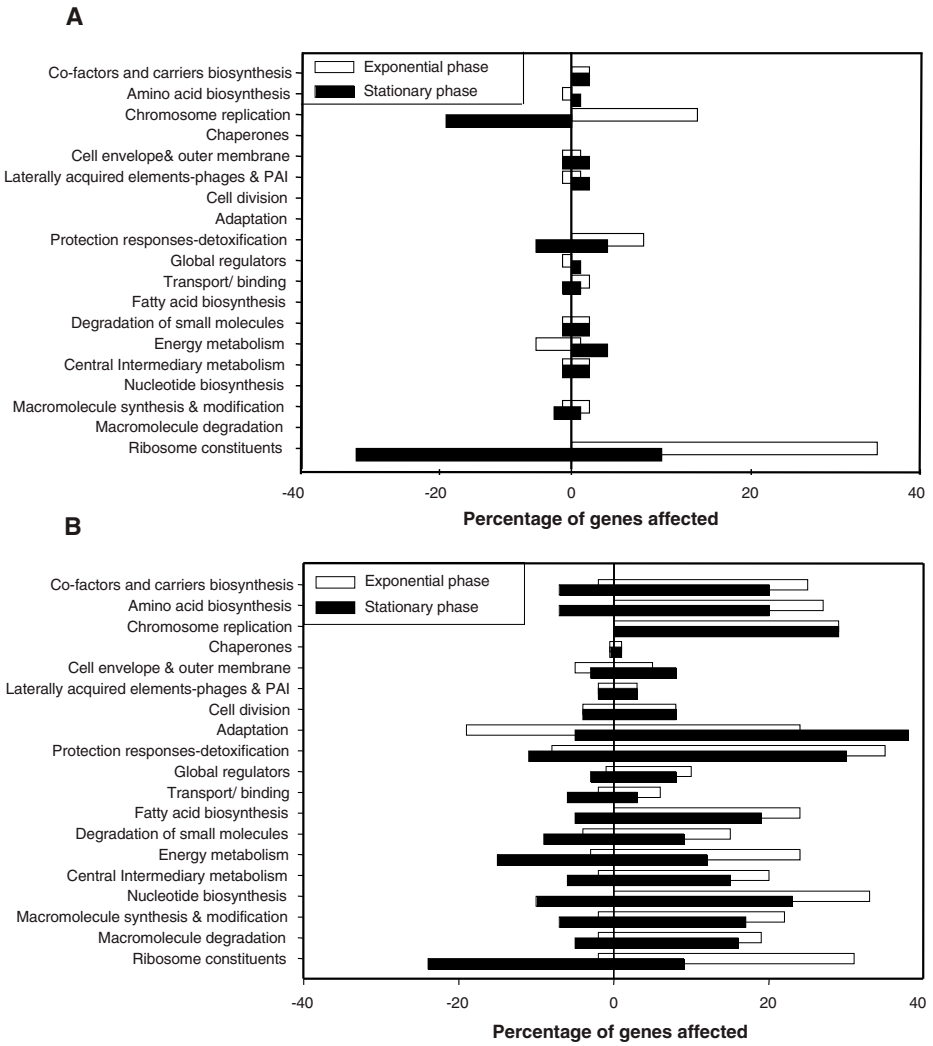

Fig. 1. The impact of plasmids pSf-R27 and pSf-R27Δ*sfh* on the transcriptome of SL1344. Data are presented for exponential (white bars) and stationary-phase (black bars) microarray data from SL1344 versus SL1344 (pSf-R27) (**A**) and SL1344 (pSf-R27) versus SL1344 (pSf-R27Δ*sfh*) (**B**). Gene categories are given at the left of each panel and are based on the Kyoto Encyclopedia of Genes and Genomics (KEGG). The histograms represent the percentage of genes in each category affected by the introduction of the plasmid.

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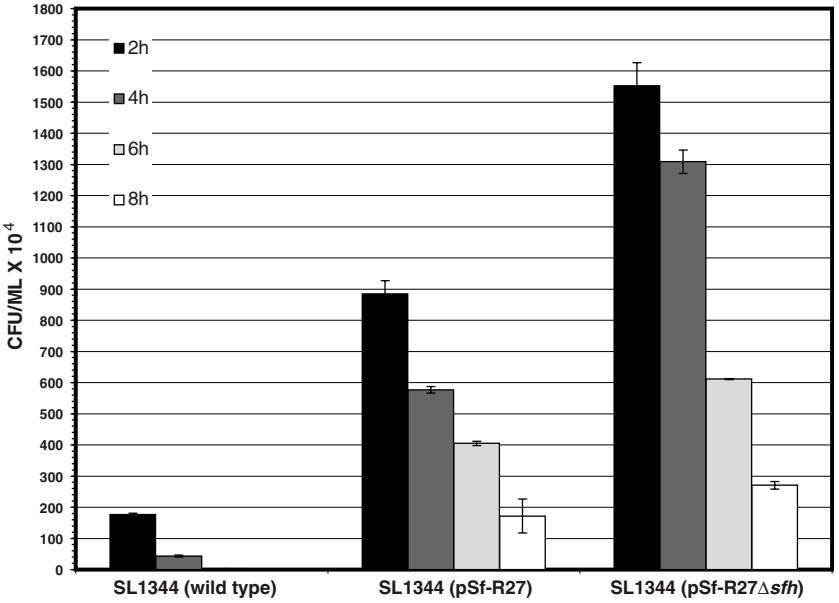

Fig. 2. The effect of plasmids pSf-R27 and pSf-R27Δ*sfh* on bacterial host survival in J774-A.1 macrophage. J774-A.1 macrophage–like cells were infected with SL1344, SL1344 (pSf-R27), or SL1344 (pSf-R27Δ*sfh*) as described in supporting online material. Bacteria were recovered at the time intervals shown, and the number of colony-forming units of each bacterial strain was determined.

Table 1. Bacterial competitive fitness measurements. Relative mean fitness values shown are with reference to the common competitor SL1344 Nal^r.

Strain	Relative mean fitness
SL1344	1.02 ± 0.04
SL1344 (pSf-R27)	0.83 ± 0.01
SL1344 (pSf-R27Δ*sfh*)	0.22 ± 0.04
SL1344 (pSf-R27Δ*sfh* pPD101)	0.47 ± 0.06
SL1344 (pSf-R27Δ*sfh* pPD101sfh)	1.30 ± 0.07
SL1344 (pUC18)	0.72 ± 0.03
SL1344 (pUCA+T)	0.47 ± 0.02
SL1344 (pUCA+T, pPD101sfh)	0.98 ± 0.02
SL1344 (pPD101sfh)	1.06 ± 0.02

reverse transcription polymerase chain reaction (figs. S1 and S2). Consistent with the changes seen in its transcriptome, SL1344 (pSf-R27Δ*sfh*) was considerably more resistant to ultraviolet radiation than either of the other strains (fig. S1) and had an enhanced level of survival in macrophage (Fig. 2). It also displayed reduced motility (fig. S2). The changes in motility and virulence were reminiscent of the phenotypes of *hns* mutants (*14*–*16*). Full motility was restored when the *hns* gene was expressed from a recombinant plasmid, and the cloned *sfh* gene also restored full motility in an *hns* mutant, consistent with the known interchangeability of these proteins (fig. S2).

Overall, these data suggested a correlation between the presence of the pSf-R27Δ*sfh* plasmid and a mild H-NS$^-$ phenotype, even though the *hns* gene on the *Salmonella* chromosome remained intact and its expression was unaltered (tables S1 to S4). Given the wide-ranging influence of pSf-R27Δ*sfh* on the gene expression profile and several phenotypes of SL1344, we tested the possibility that the introduction of this plasmid might influence the competitive fitness of the bacterium. The presence of pSf-R27 had a mild negative effect on the fitness of SL1344, whereas the mutant plasmid pSf-R27Δ*sfh* strongly reduced bacterial fitness (Table 1). H-NS is known to target A+T-rich sequences in the *Salmonella* genome (*15*, *16*). The *hns*-like phenotype of the exconjugants harboring pSf-R27Δ*sfh* suggested that the A+T-rich plasmid might influence the global gene expression pattern of the bacterium—and hence its fitness—by titrating H-NS, a protein that is closely related to Sfh. Both proteins bind to pSf-R27Δ*sfh* (fig. S3) and have strikingly similar DNA structural requirements for binding (*10*, *11*). They also bind to the regulatory region of the *ssrA* virulence regulatory gene (fig. S4) and other genes identified in this study as responding to the presence of pSf-R27Δ*sfh*. We tested this hypothesis by cloning the DNA fragment encompassing the Sfh/H-NS binding region from the *ssrA* promoter into plasmid pUC18. Such binding regions consist of a nucleation site from which H-NS can polymerize along the DNA (*13*). This construct caused a strong reduction in SL1344 fitness, and the subsequent introduction of a compatible recombinant plasmid (pPD101sfh) expressing the Sfh protein restored fitness (Table 1). This showed that the reduction in fitness was due to the presence of multiple copies of the H-NS/Sfh binding region and did not require any property that was specific to the pSf-R27Δ*sfh* plasmid.

We propose that *sfh* is a stealth gene that allows the A+T-rich pSf-R27 plasmid to enter new bacterial hosts with a minimal impact on global gene expression patterns and fitness, ensuring the future competitiveness of the new plasmid-host combination. This strategy has the effect of smoothing the horizontal transmission of genetic information within and between bacterial populations, as evidenced by the presence of *hns*-like genes on other plasmids (*17*–*20*) and on other horizontally acquired A+T-rich DNA elements such as pathogenicity islands that are known to bind H-NS (*20*, *21*). The positive effect on competitive fitness of adding the *sfh* gene to commonly used cloning vectors has obvious biotechnological implications.

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Materials and Methods
Figs. S1 to S4
Tables S1 to S6
References

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Picobiliphytes: A Marine Picoplanktonic Algal Group with Unknown Affinities to Other Eukaryotes

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Environmental sequencing has revealed unimagined diversity among eukaryotic picoplankton. A distinct picoplanktonic algal group, initially detected from 18*S* ribosomal DNA (rDNA) sequences, was hybridized with rRNA λ-targeted (rRNA-targeted) probes, detected by tyramide signal amplification–fluorescent in situ hybridization, and showed an organelle-like body with orange fluorescence indicative of phycobilins. Using this fluorescence signal, cells were sorted by flow cytometry and probed. Hybridized cells contained a 4′,6′-diamidino-2-phenylindole–stained organelle resembling a plastid with a nucleomorph. This suggests that they may be secondary endosymbiotic algae. Pending the isolation of living cells and their formal description, these algae have been termed picobiliphytes.

Molecular tools applied to DNA retrieved from marine microorganisms have revealed considerable diversity among the smallest eukaryotic cells (*1*–*3*), paralleling that found among marine prokaryotes. Together with a high taxonomic diversity, the finding of many sequences unrelated to those of known organisms was an additional striking feature of these first studies. Clone libraries for the eukaryotic 18*S* ribosomal RNA (rRNA) gene were constructed at different times from fractionated water samples (using a filter pore size of 3 μm) from three coastal sites (*4*–*6*), and additional libraries were established from three more open-water sites (*7*, *8*) (table S2). A particular group of sequences was recovered irregularly throughout the year (*8*) (table S2) and referred to as the "Rosko II" group from partial 18*S* sequence phylogenies from these sites (*4*–*6*). Analyses of full-length sequences (*8*) reveal that they form an independent phylogenetic group among major eukaryotic taxa (Fig. 1), (*9*, *10*), which we have tentatively called picobiliphytes. Our complex iterative Bayesian analyses (*8*) indicate that the picobiliphytes are an independent lineage, possibly having a weak sister relationship with the cryptophyte/katabletablepharid clade, although its true sister group is difficult to assign using a single gene phylogeny. The inability to assign an affinity of the picobiliphytes to any other major eukaryotic group (table S1) in the eukaryotic 18*S* rDNA tree was confirmed with the Kashino-Hasagawa test (*8*) (table S3). Their deep branching suggests that they probably deserve a taxonomic rank of division or phylum.

Picobiliphytes consist of at least three different clades (Fig. 1), for which we were able to identify two signature sequences: PICOBI01 (5′-GCGTGATGCCAAAATCCG-3′) and PICOBI02 (5′-ATATGCCCGTCAAACCGT-3′), which target most picobiliphytes (tables S4 and S5). They have two or more mismatches with all available GenBank sequences from cultivated protists (tables S4 and S5) and do not display any fluorescence when hybridized to a variety of algal strains from the Roscoff Culture Collection (*8, 11*) (table S6). In addition, they match a set of five additional environmental 18*S* rDNA partial sequences: four from the western North Atlantic (*12*) and one from a mid-Atlantic estuary (Barnegat Bay, New Jersey), extending the possible distribution of the picobiliphytes. These probes enabled us to determine, by microscopy after tyramide signal amplification–fluorescent in situ hybridization (TSA-FISH) (*13*), the gross morphology of fixed cells from the Roscoff coastal site (Fig. 1 and fig. S1). The morphology of other unknown marine protist groups was also determined by Massana *et al*. (*14*), using probe methods.

Picobiliphytes are unicellular, slightly oblong, and approximately 2 × 6 μm (n = 9 cells) and were recovered in the picoplankton size fraction of our water samples because they probably passed though the 3-μm pores in the filter by way of their smallest dimension. Thus, we have referred to them as picoplankton. One remarkable feature is the presence of an organelle-like structure having orange autofluorescence when excited with blue light under epifluorescence microscopy (Fig. 1), a structure similar to that of phycobiliprotein-containing rhodophytes and cryptomonads (fig. S1). These pigments, in contrast to chlorophylls, are water-soluble (*15*) and thus not removed by the TSA-FISH alcohol dehydration steps. Moreover, any chlorophyll remaining after alcohol dehydration fluoresces yellow, not orange, under blue light (fig. S1). Thus, picobiliphytes probably have a phycobiliprotein-containing organelle, most probably a plastid. Another distinctive feature is a small body that is stainable with the nucleic acid–specific dye DAPI (4', 6-diamidino-2-phenylindole), distinct from the main nucleus and consistently seen in close proximity to the presumed plastid (Fig. 1, fig. S1).

Picobiliphyte sequences have been found in a variety of marine systems, including the European coast (*8*), the North Atlantic (from GenBank Blast searches), and the Arctic Ocean (*7*). A detailed look at their abundance, applying TSA-FISH in size-fractionated (<3 μm) seawater samples from the English Channel, revealed that picobiliphytes occurred mostly in fall and winter and were not detected by FISH in summer, although their sequences were occasionally detected in summer clone libraries (tables S2 and S8). Their concentration, up to 80 cells ml^{-1}, accounted for about 1.6% of the total picoeukaryote cell counts at one coastal station in the English Channel and corresponded to a major proportion (33 to 81%) of orange-fluorescing picoeukaryotic cells detected by blue laser flow cytometry (tables S7 and S8). In one particular sample, cells exhibiting this fluorescence were sorted by flow cytometry and subsequently hybridized by TSA-FISH with our two probes (*8*) (table S7). We found that 48 to 61% of the sorted cells were labeled with probes PICOBI01 and PICOBI02, suggesting that the picobiliphytes may constitute a substantial proportion of the orange-fluorescing eukaryotic picoplankton previously thought to be cryptophytes (*16*). The fact that our cells could have been sorted and enriched with a phycobilin pigment signature detected with flow cytometry further supports the contention that they actually possess such pigments (*15*, *16*).

The inferred presence of a phycobiliprotein-containing plastid in picobiliphytes is in good agreement with their putative sister relationship to cryptophytes and katablepharids, the first of which contain phycobiliproteins. Whereas cryptophytes are common in the marine nanophytoplankton, pico-sized cryptophytes are not as abundant, as judged by their relative frequency in clone libraries; and where found, their 18*S* rDNA sequence places them as an independent lineage within the nano-sized cryptomonads (*5*, *6*). There are also small cell forms among the red algae, such as the marine Porphyridiales, but our group does not belong to the rhodophytes, based on our phylogenetic analysis. Cryptophytes are a well-known example of a secondary endosymbiosis of a rhodophyte, which brings phycobilin pigments to the new host cell. Because picobiliphytes are sister to the cryptophyte/katabletablepharid clade in most of our complex Bayesian analyses (*8*) (Fig. 1), it would be most parsimonious to assume that our group is a secondary endosymbiotic alga. The small

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body stainable with the nucleic acid–specific dye DAPI (Fig. 1) may be a DNA-containing nucleomorph, similar to that found in cryptophytes and chlorarachniophytes (*17*), supporting the idea that picobiliphytes are another secondary endosymbiotic algal group (*18*).

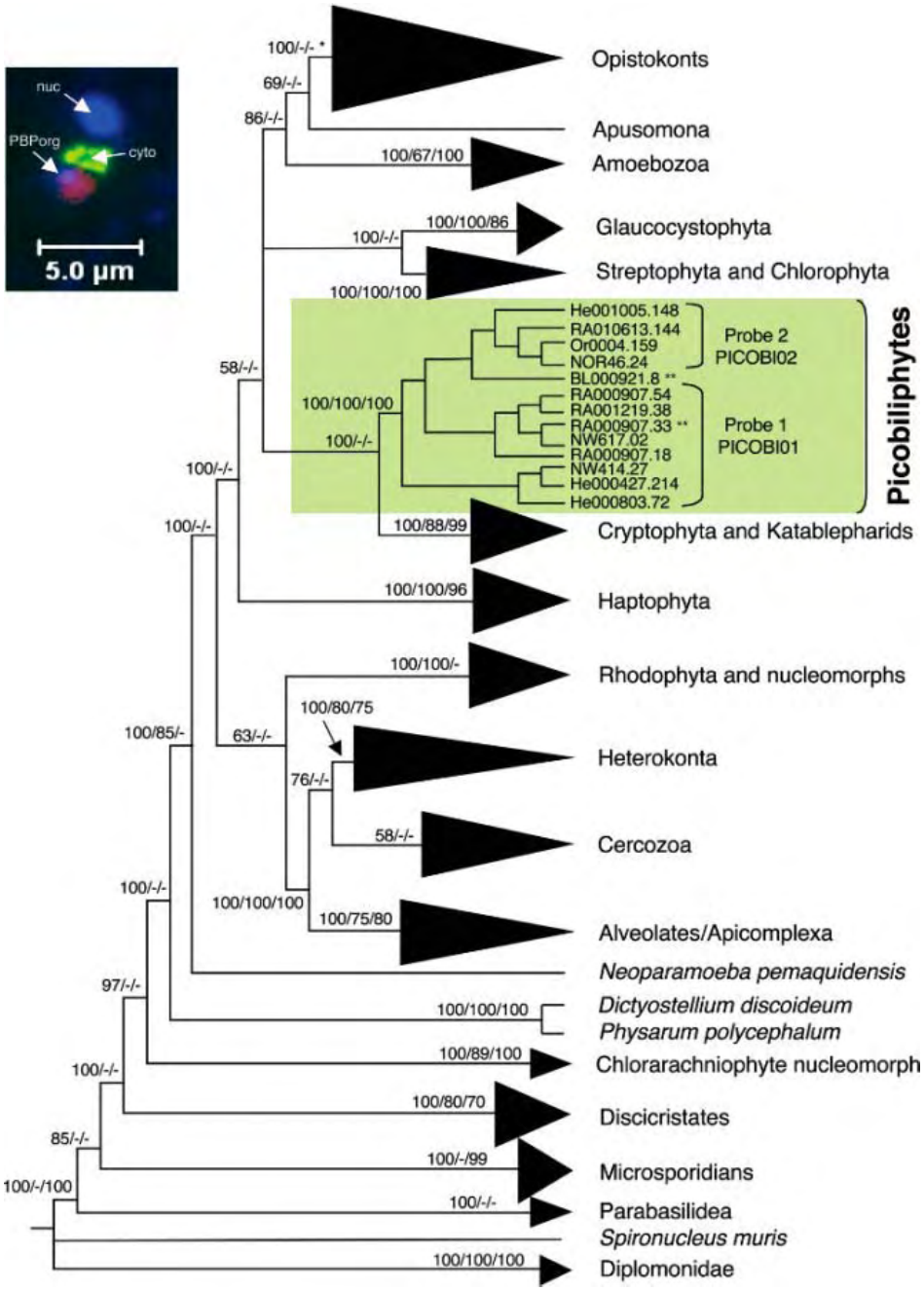

Fig. 1. Phylogenetic trees were reconstructed from full-length 18*S* rRNA sequence data listed in table S1 and inferred with Bayesian analysis from two parallel runs, each with one million generations with six chains and increased temperature between the chains to facilitate exchange between the chains (*8*). This tree is the 50% majority-rule tree of the last 100 trees saved from one of the parallel runs. Support for each node was also determined with 100 replicated bootstrap analyses of weighted maximum parsimony and neighbor-joining analyses. Nodes supported by bootstrap or posterior probability values above 50% are labeled for the three methods used (MrBayes/maximum parsimony/neighbor-joining). If a clade was not supported by a method, it is indicated by a dash. The asterisk indicates that internal major clades were supported by 100 posterior probabilities from the MrBayes analysis. PICOBI01 and PICOBI02 are specific for the sequences belonging to the three clades as bracketed. (**Insert**) Picture of a cell targeted by the probe PICOBI02 (specific for picobiliphyte clade 2) from the Roscoff ASTAN sampling site on 26 September 2001. Arrows point to the DAPI-stained nucleus (nuc) in blue, to the green fluorescence from probe-specific labeling of the small subunit rRNA in the cytoplasm (cyto), and to the red autofluorescence from the phycobiliprotein-containing organelle (PBPorg). Double asterisks indicate sequences not recognized by the probes.

Kleptoplastidy is another possibility, such as in the katablepharids (*19*, *20*), which along with the cryptophytes are the picobiliphytes' purported sister group. However, kleptoplastidy is unlikely in such small organisms. In the absence of living cells to follow through cell division, we screened filtered 3-µm–fractioned water for cells that hybridized with our probes, using a Chem*Scan* solid-phase cytometer (*8*) (fig. S2). We never encountered positive cells without a plastid on the filters scanned by the laser, which implies that the cells are predominately pigmented, so kleptoplastidy does not seem very likely.

Are the picobiliphytes representatives of another red algal secondary endosymbiosis, such as chromo-alveolates, in the broad sense, or do they have kleptoplastids? Without living cells, the status of their endosymbiosis and a formal description will remain unresolved. Nevertheless, picobiliphytes are pigmented and thus contribute to primary production. Molecular analysis confirms that they are a eukaryotic group that should be recognized at the phylum or division level, without any real indication of their sister group. We found that they are well represented in polar and cold temperate coastal marine ecosystems, as judged from their appearance in clone libraries and preliminary FISH data. The putative presence of a DNA-containing body in the purported plastid places them in an intriguing position in the study of plastid reduction to organelles.

Within the past 15 years, four algal classes have been described from the picoplankton [see (*5*) for details], and picobiliphytes represent another division or phylum. The phylogenetic analysis indicates that they are a highly diverse group, composed of at least three distinct clades. The temporal and spatial scales at which they occur, as inferred from molecular data, indicate that they could make up a substantial picoplankton fraction under certain conditions. The existence of small, sometimes rare, organisms is only now being recognized, and their role in ecosystem function is unknown, but they probably act as reservoirs of genetic capacity that are activated under specific conditions. The discovery of picobiliphytes and their apparent widespread distribution and contribution to marine protist assemblages highlight the imperative of understanding biodiversity before its loss on a global scale.

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The National Institute of Environmental Health Sciences of the National Institutes of Health is seeking an exceptional candidate to fill the position of Director, Division of Intramural Research. The incumbent of this position will direct laboratory and clinical research through 16 laboratories and branches with approximately 480 employees and 270 trainees. The Division is organized into four scientific programs, including the Clinical Research Program, which highlight the areas of research excellence of NIEHS. These programs are highly interrelated, interactive and synergistic. Using the interdisciplinary biomedical research approach, the mission of the DIR is to contribute to the basic understanding of biological and chemical processes, understanding of the contributions of environmental agents to human disease and dysfunction and to the underlying mechanisms of environmentally associated diseases. The Director, DIR, also serves as a principal advisor to the Institute Director on scientific affairs involving interdisciplinary biomedical research programs; develops and recommends policies for the execution of research programs; determines effectiveness of current programs and recommends policies for new programs; and develops new and revised programs to meet national environmental health needs.

Candidates must have either an M.D. or Ph.D. or equivalent degree in a discipline relevant to environmental health science. Salary is commensurate with level of experience. Please forward questions regarding the position to:

Dr. Ebony Bookman
National Institute of Environmental Health Sciences
P.O. Box 12233, Maildrop B2-01
Research Triangle Park, NC 27709
919-541-3201
email: bookmane@mail.nih.gov

Interested persons should send a curriculum vitae by **March 15, 2007**, to:

Ms. Stephanie Jones (DIR-07-01)
National Institute of Environmental Health Sciences
P.O. Box 12233, Maildrop NH-01
Research Triangle Park, NC 27709
919-541-7913
e-mail: jones17@niehs.nih.gov

U.S. Department of Health and Human Services
National Institutes of Health

The NIH is dedicated to building a diverse community in its training and employment programs.

DHHS and NIH are Equal Opportunity Employers

Tenure/Tenure-Track Investigator in HIV Research
Laboratory of Molecular Microbiology

NIAID's Laboratory of Molecular Microbiology (LMM) is seeking a viral immunologist for a tenure-track (assistant professor) position to develop an independent research program focusing on the immune responses to primate lentiviruses. Candidates should have an M.D, Ph.D. or D.V.M degree. The ideal candidate will have extensive research accomplishments in viral immunology in the context of primate lentivirus pathogenesis and vaccine development.

This position provides access to modern research facilities and technologies including flow cytometry, DNA sequencing, oligonucleotide and peptide synthesis, confocal/light microscopy, microarrays, proteomics, and mass spectrometry. A competitive federal salary and benefits package will be provided, along with an operating budget for equipment, supplies, and staff.

Questions about this position can be sent to **Dr. Malcolm Martin at malm@nih.gov**. To apply, submit a curriculum vitae, bibliography and 2-3-page description of a proposed research program, preferably via e-mail to **Ms. Felicia Braunstein at braunsteinf@niaid.nih.gov**. In addition, three letters of recommendation must be sent to **Ms. Felicia Braunstein, NIAID, NIH; Bldg. 10, Rm. 4A31, MSC-1356; Bethesda, MD 20892-1356**. Applications must be received by **February 28, 2007**. Please note search **#007** in all correspondence. Applicants will be notified by e-mail or phone phone when their applications are received and complete.

Please see our web site at **http://www.niaid.nih.gov/dir/labs/lmm.htm** for information about current LMM principal investigators and their research interests.

Tenure/Tenure-Track Position
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories, Hamilton, Montana

The Laboratory of Persistent Viral Diseases (LPVD), Rocky Mountain Laboratories, NIAID, NIH, DHHS, in Hamilton, Montana, seeks applicants for a tenured or tenure-track position (full to assistant professor equivalent) to conduct independent research on host immune or inflammatory responses in neuropathogenic viral diseases. Candidates with a background in adaptive or innate immunity, including neuroinflammation and gliosis are preferred; those interested in neurobiology, biochemistry or pathogenesis of CNS infections are also encouraged to apply. Candidates must hold a Ph.D., D.V.M, or M.D. degree and have a minimum of 3 years of relevant postdoctoral experience. Candidates must be able to develop an independent research program, supervise staff and fellows, and collaborate with other LPVD researchers working on CNS viral or prion diseases.

Rocky Mountain Laboratories' state-of-the-art facilities include an operational BSL-3 facility, a BSL-4 lab and animal facility nearing completion, and in-house core facilities for genomics, electron microscopy, and flow cytometry. Research programs focus on prions, murine retroviruses, HIV, flaviviruses, and numerous pathogenic prokaryotic organisms. The lab is located in the scenic Bitterroot Valley of western Montana with easy access to some of the finest outdoor recreational opportunities in North America. Additional information on the position may be obtained by contacting Dr. Bruce Chesebro at bchesebro@niaid.nih.gov.

Application Process: Salary depends on degree and qualifications. To apply, submit a curriculum vitae and bibliography, including a list of your five most significant papers, and a 2-3-page description of a proposed research program, via e-mail to **Ms. Felicia Braunstein at braunsteinf@niaid.nih.gov**. In addition, three letters of recommendation must be sent directly from the referees to **Ms. Felicia Braunstein, Committee Manager, NIAID/NIH; 10 Center Drive, Bldg. 10, Rm.4A31, MSC-1356; Bethesda, MD 20892-1356**. Applications must reference **AD #010** and must be received by **March 9, 2007**. Applicants will be notified when their applications are received and then completed. All information provided by applicants will remain confidential.

WWW.NIH.GOV

Tenure-Track and Tenured Investigator Positions in Systems Immunology and Infectious Disease Modeling

The National Institute of Allergy and Infectious Diseases (NIAID), Division of Intramural Research (DIR) is seeking several outstanding individuals for its new Program in Systems Immunology and Infectious Disease Modeling (PSIIM).

Modern technology allows the analysis of immune responses and host-pathogen interactions at multiple levels—from intracellular signaling networks, to individual cell behavior, to the functioning of a tissue, organ, or even whole organism. The challenge is not only to collect large amounts of data, but also to organize it in a manner that enhances our understanding of how the immune system operates or how pathogens affect their hosts. To do this, we need to develop detailed quantitative models that can be used to predict the behavior of a complex biological system. These models can help to explain the mechanisms underlying physiological and pathological responses to infection or vaccination, which can then be exploited to design better therapies or vaccines.

Achieving this goal requires an interdisciplinary effort and to this end the PSIIM will be organized as an integrated team of scientists and support staff with expertise in computational biology, bioinformatics, proteomics, cell biology, immunology, and infectious diseases, rather than as a group of independent laboratories. These teams will have access to the latest technology for gene-expression profiling, high-content screening of RNAi libraries for the discovery of pathway components, imaging tools, cores for the genetic manipulation of animals and for proteomic analysis, and a substantial computer infrastructure. BSL-3 facilities for working with high priority pathogens will also be available.

Although the PSIIM has been established within NIAID and has an immune system/infectious disease focus, we expect it to foster the growth of systems biology efforts at other NIH Institutes, primarily through the development of new software tools for complex systems modeling and methods for high-throughput screening. Thus, PSIIM team members are expected to interact extensively with other NIH scientists and with extramural groups in the U.S. and abroad who share our interest in a systems approach to biology.

The PSIIM is now recruiting for tenure-track or tenure level team leader appointments in three key areas:

Computational Biology: The incumbent will lead a group focused on the development and improvement of software tools for multiscale modeling and simulation that can be used by the PSIIM as well as by biologists interested in subjects other than immunity or infectious diseases. The ideal candidate will have a strong background in mathematics, physics, and computer programming, and a clear desire and ability to interact with and support the efforts of biologists. A demonstrated ability to generate computer software tools for biological modeling will be a strong plus.

Molecular/Cell Biology: The incumbent will lead a group involved in the design, implementation, and interpretation of screening efforts to identify and determine the interactions among the components in signaling networks that could then be modeled using the software generated by the computational biology team or obtained from other sources. Discovery tools such as gene arrays, high-content image-based screens using RNAi methods, various protein-protein hybrid screening methodologies, and optical imaging are expected to be key elements in the efforts of this group. A strong background in basic cell biology and molecular biology with experience in analysis of protein-protein interactions, signaling, and/or gene regulation is required. Expertise in large-scale screening is highly desirable.

Infectious Diseases: The incumbent will be responsible for developing novel approaches to systems-wide analysis of the interaction of infectious agents and their hosts. These may include the use of gene-expression signatures, the production of gene-modified animals, the development of methods for in vivo testing of the predictions of models, and the use of sophisticated imaging and other tools for probing the interaction of pathogens and host cells in vitro. A strong background in viral and/or bacterial infectious diseases and cell and molecular biology are necessary; training in the immunology of infectious diseases and substantial bioinformatics experience are highly desirable.

These positions and the research activities they conduct are fully funded by the intramural research program of NIH. Each team leader is expected to build a working group consisting of postdoctoral fellows, staff scientists, technicians, and students. The team leaders will work with the program director to help set the goals for the PSIIM and to determine how best to reach these goals as an integrated group. To ensure appropriate career trajectories for those joining the PSIIM team, the NIH has modified its tenure decision policies to encourage and account for contributions made in such a team science setting. Applicants should be seeking a difficult challenge in which creativity, technical expertise, and a strong desire to achieve in a team environment are critical for success.

Interested candidates may contact **Dr. Ronald Germain, Program Director, PSIIM, DIR, NIAID at 301-496-1904 or rgermain@niaid.nih.gov** for additional information about these positions.

To apply, submit your curriculum vita, bibliography, and detailed statement of how you can contribute to the success of the PSIIM program to **Felicia Braunstein at braunsteinf@niaid.nih.gov**. In addition, three letters of reference must be sent directly from the referee to **Dr. Robert Hohman, Chair, NIAID Search Committee, c/o Ms. Felicia Braunstein, DIR Committee Management Team Lead, 10 Center Drive, MSC 1356, Building 10, Room 4A31, Bethesda, Maryland 20892-1356**. Completed applications **MUST** be received by **February 16, 2007** for computational biology, and **March 16, 2007** for Molecular/Cell Biology as well as for infectious diseases. Please refer to ad **#012 for computational biology, #013 for molecular/cell biology, and #014 for infectious diseases** on all correspondence. Further information on these positions and guidance on submitting your application are available at **http://healthresearch.niaid.nih.gov**. For more information about the NIAID systems biology program, please visit **http://www.nih.gov/catalyst/2006/06.09.01/page1.html**

Dean – College of Engineering, Northeastern University

Northeastern University invites applications and nominations for the position of Dean, College of Engineering. The Dean reports to the Provost and has direct responsibility for all academic, administrative and fiscal operations in the College, including its cooperative education programs. The Dean will provide leadership for the College's strategic and academic planning, will drive institutional advancement and alumni relations for the College, and further advance its strong relationships with government agencies, industry and the community. With current external research related funding in excess of $40M, the College is home to two NSF-funded centers; the Center for Subsurface Sensing and Imaging Systems – an ERC, and the Center for High-Rate Nanomanufacturing – an NSEC, and a number of internationally acclaimed research clusters. The Dean is expected to play an active role in attracting new major interdisciplinary centers of excellence to the College. Such initiatives should include cross-disciplinary programs within the college as well as activities with other colleges and units throughout the University.

The successful candidate should have an outstanding record of scholarly and professional accomplishments that would merit tenure with the rank of Full Professor in one of the academic departments in the College of Engineering. Strong candidates from National Labs or industry will also be considered.

Northeastern University, founded in 1898, is a large, private and national research university offering a comprehensive range of undergraduate and graduate programs leading to degrees through the doctorate in six undergraduate colleges, eight graduate schools. In 2003, U.S. News & World Report ranked Northeastern number one in the country among programs that combine classroom learning with real-world experience. Located in the heart of Boston, near the financial district and in the biomedical research corridor, the University enjoys close ties to the numerous high technology industries that flourish in the region.

Applications and nominations should be forwarded, in confidence, to:

Alberto M. Pimentel - Vice President
Edward W. Kelley & Partners
1111 Corporate Center Drive, Suite 106, Monterey Park, CA 91754
alberto.pimentel@ewkp.com; Tel: 323-260-5041

Applicants should submit a complete resume, and the names and addresses of three references. Electronic submission is encouraged. Nominators are encouraged to submit names by January 31, 2007. The search will continue until the position is filled.

Potential applicants are invited to discuss the opening with the Committee Chair c/o Deborah K. Berwaldt; 617-373-4517 (voice); 617-373-8589 (fax); d.berwaldt@neu.edu

Northeastern University is an Equal Opportunity, Affirmative Action Educational Institution and Employer, Title IX University and particularly welcomes applications from minorities, women and persons with disabilities.

Northeastern UNIVERSITY

http://www.neu.edu

Founded in 1977, Lipoid is a globally leading manufacturer of lecithins and phospholipids for the pharmaceutical, cosmetic, and dietetic industries. Lipoid is headquartered in Germany and privately held. Our high-grade products are scientifically developed in Lipoid's laboratories, manufactured on an industrial scale, and marketed globally.

Lipoid is seeking a

Director of Sales

in order to deepen our market penetration in North America.

Responsibilities:

- Sell Lipoid's products in North America to the processing industries
- Provide technical consulting for the application of our products
- Make presentations at international trade shows
- Compose scientific documents for internal and external use
- Review and analyze the pertinent scientific literature

After initial training in our German headquarters, the person hired will be located in our US office in New Jersey.

Skills:

- A scientific degree (B.S., Master's, or Ph.D.) and/or business degree is required
- Successful experience in the market for pharmaceutical ingredients is advantageous
- Lipoid requires integrity, entrepreneurial spirit, flexibility, readiness to undertake significant traveling, and commitment to nurture long-lasting customer relationships

Lipoid will provide the employee with a flexible and creative environment, a competitive salary (including retirement benefits and medical insurance), and opportunities for career advancement.

If interested, please submit your resume by mail or email to:

Lipoid

Lipoid LLC • 744 Broad St. Suite 1801 • Newark • NJ 07102
att. Matthias Rebmann • mrebmann@lipoidllc.com
www.lipoid.com • Equal opportunity employer.

ASSISTANT PROFESSORS
DEPARTMENT OF PHYSIOLOGY
Texas Tech University
Health Sciences Center

The Department of Physiology at TTUHSC invites applications from scientists for several tenure-track positions at the rank of Assistant Professor in the Medical School. Exceptional candidates may be considered for more senior positions. The successful candidates will be expected to develop and/or maintain an independent program of research with external funding in the general areas of Cell Physiology and/or Molecular Biophysics. Individuals interested in structure, function or pathophysiology of membrane proteins will be given special consideration. Participation in medical and graduate training is expected.

Dr. Luis Reuss has been recruited as Chair of Physiology and, in conjunction with other faculty in the Medical School and the University, will develop an Institute for the Study of Membrane Proteins.

Review of applications will start in January 2007. Applicants should send electronically a current CV, an outline of research plans, and the names and addresses of three or more potential referees to:

Ariel Escobar, Ph.D.
[Physiology.Search@ttuhsc.edu]
Chair, Physiology Search Committee
Department of Physiology
Texas Tech University
Health Sciences Center
Lubbock, TX 79430-6551

Texas Tech University Health Sciences Center is an EEO/AA/ADA Employer.

The Masdar Institute of Science and Technology, Abu Dhabi, United Arab Emirates
Provost, Vice President for Business Operations, and Vice President for Research

The Masdar Institute of Science and Technology, a new and independent non-profit, tax-exempt research and educational institution, is being founded with the assistance and advice of the Technology and Development Program at Massachusetts Institute of Technology (MIT). Initially offering graduate-level courses in Abu Dhabi for a highly select student population, The Masdar Institute is dedicated to premier engineering research and the provision of a definitive, research-driven education. The goal of the Institute is to develop, over a period of years, indigenous research and development capacity in Abu Dhabi, addressing issues of importance to the region in areas as critical as: renewable energy, sustainability, environment, water resources, systems engineering and management, transport and logistics, and advanced materials. The mission and government supported mandate of The Masdar Institute is to provide qualified men and women in the region with the opportunity to obtain graduate degrees in these critically important and globally relevant technical fields.

Provost is the Chief Academic Officer of the Institute and serves as the executive officer. The Provost is involved in all major policy decisions of the Institute and works with the President to provide leadership to the institution to create a 21st century graduate institution with a clear focus on student success through academic excellence, strong institutional partnerships, scholarship, meaningful service to the region, and effective resource utilization.

Qualifications: The successful candidate must possess impeccable character and personal integrity and have an earned doctorate coupled with a record of substantial scholarly achievement meriting appointment as a tenured full Professor in one of the academic departments; significant academic administrative experience and seasoned leadership skills; proven credentials in management, including budget development and academic, administrative, and strategic planning, as well as evidence of successful experience in the areas of curriculum development and outcomes assessment and with integrating information technology for academic programs and distance learning; demonstrated skills and commitment to cultivating excellence in scholarship, teaching, and public services; and demonstrated commitment to cultural diversity and shared governance. The successful candidate must be a collaborator and team player who is skillful in promoting a collegial work environment and can foster an environment that attracts, retains, and engages faculty and staff of the highest quality.

Vice President for Research to lead a vibrant and complex interdisciplinary research and academic institution of higher learning. This individual is expected to work closely with the various levels of administration (deans, chairs, principal investigators and center/institute directors), faculty and student governance, staff councils, regional centers, and national labs. Will have direct responsibility for creation and review of large interdisciplinary research centers and institutes; faculty research startups and retention packages and seeding of initiatives and internal research programs; institutional-wide research affiliations/memberships; private/public partnerships that impact research; addressing the research agenda and mission of the Institute; research compliance, policies, ethics and conflict of interest; management of research space, animal care, and human subjects; material transfer and confidentiality agreements; liaison to sponsor negotiations and export controls; intellectual property portfolio; sponsored program data and office of contracts and grants, institutional research marketing publications, and institutional workshops on proposal writing and funding opportunities.

Qualifications: Seasoned academic administrator with at least 10 years of leadership experience in research operations. The ideal candidate will have a demonstrated ability to provide strategic leadership to collaborative research efforts as well as a track record of creating a working environment that rewards new ideas and innovation, builds collaborations, encourages and nurtures teamwork, and promotes diversity. The applicant should be familiar with the land grant research university environment as well as current trends in technology transfer, patenting and licensing, research administration best practices, and compliance regulations. Applicant should be perceived as a visionary leader with demonstrated success in obtaining extramural funds and fundraising; evidence of a thorough understanding of federal agencies, national and international research organizations, institutional partnerships and collaborations, public and private organizations, evidence of success in managing others; demonstrated research excellence within an academic or related setting; an appreciation for a broad range of research and scholarship; experience with research infrastructure, contract and grant administration, national and state policies; information systems; consensus building; measures of accountability; community outreach; corporate opportunities and interests; strategic planning; evidence of successful decision-making in complex environments and situations; budgeting; and developing prospecting plans. An earned doctorate is prerequisite.

Vice President for Business Operations who reports to the President and Provost of the Institute and who will direct and oversee system-wide management of the Institute's business operations at all of the Institute's facilities. Institute business operations include human resources, information technology, procurement, facilities, research administration, compliance and audit, laboratory administration, security and public safety. This individual will lead and manage Institute policies, systems and procedures, plan and supervise the departmental budget, and serve as a liaison, both internally and externally, for the various business operations of the Institute.

Qualifications: The successful candidate should have strong leadership skills, the ability to work successfully with a broad range of constituencies, and a demonstrated track record of identifying and implementing administrative process changes and improvements. A Master's degree and a minimum of 5-10 years' general management experience in a large, complex business, academic, government or not-for-profit organization is required. Leadership experience in private, not-for-profit higher education institutions is highly preferred. Candidates should be able to demonstrate an in-depth knowledge of the full range of issues addressed by an administration and finance executive in the graduate education setting. Proficiency and knowledge of business practices and organizational leadership in changing environments is essential and candidates must have superior oral and written communication skills, excellent motivational techniques, sophisticated computer applications, and experience in management, financial analysis, and legal aspects of higher education partnerships and affiliations.

Application Submittal Information: The Technology and Development Program at Massachusetts Institute of Technology is assisting in the search. Initial screening of applications will begin immediately. Applications must be received electronically by the Search Committee Co-Chairs by March 1, 2007. Submissions should include the applicant's name, present position, postal and e-mail addresses, and telephone numbers. Application materials should include a description of how the candidate's experiences match the position requirements; a detailed curriculum vitae and summary document; and specific contact information for a minimum of three professional references familiar with the candidate's qualifications and experience.

Materials must be submitted electronically on or before **01 March 2007** as a MS Word attachment to:

Professor Fred Moavenzadeh, Co-Chair
Search Committee for The Masdar Institute of Science and Technology
Technology and Development Program
Massachusetts Institute of Technology
Email: TDPmail@mit.edu

Mr. Sultan Al Jaber, Co-Chair
Search Committee for The Masdar Institute of Science and Technology
Office of Institutional Development
Abu-Dhabi, United Arab Emirates
Email: saljaber@mubadala.ae

POSTDOCTORAL TRAINING in CANCER RESEARCH

Louisiana Cancer Research Consortium IN NEW ORLEANS

The Louisiana Cancer Research Consortium is supporting major expansions of the Cancer Research Programs at Tulane University Health Sciences and Louisiana State University Health Sciences Centers in New Orleans. Outstanding postdoctoral positions are available with researchers in Basic Science, particularly with a focus on cancer genetics, immunology or cell signaling. Please send letters of interest, along with CVs, by e-mail to postdoc@LCRC.info, or contact mentors individually. Some information on the Consortium is available at www.LCRC.info. NRSA-eligible candidates are preferred.

Brian Barnett, M.D. – *tumor immunotherapy*
Matthew Burow, Ph.D. – *estrogen receptor signaling and breast cancer*
Andrew Catling, Ph.D. – *MAP kinase and scaffold control of cell motility*
Yan Cui, Ph.D. – *tumor immunology and immunogene therapy*
Srikanta Dash, Ph.D. – *hepatocellular carcinoma*
Prescott Deininger, Ph.D. – *mobile elements and genetic instability*
Melanie Ehrlich, Ph.D. – *DNA methylation*
Astrid Engel, Ph.D. – *mutational activity of human mobile elements*
Erik Flemington, Ph.D. – *Epstein-Barr Virus and tumor biology*
Ed Grabczyk, Ph.D. – *intrinsic genetic instability*
Carl Gregory, Ph.D. – *adult stem cell signaling*
Arthur Haas, Ph.D. – *ubiquitin- and ISG1-dependent regulation*
Michael Hagensee, M.D., Ph.D. – *immunology of human papillomavirus infection*
Charles Hemenway, M.D., Ph.D. – *leukemia biology*
Steven Hill, Ph.D. – *melatonin/G-protein coupled receptors and breast cancer*
Tadahide Izumi, Ph.D. – *DNA damage and repair*
S. Michal Jazwinski, Ph.D. – *aging and cancer*
Frank Jones, Ph.D. – *EGF receptors*
Shahriar Koochekpour, M.D., Ph.D. – *tumor-stromal interaction and AR signaling in prostate cancer*
Iris Lindberg, Ph.D. – *activation of matrix degrading enzymes*
Laura Levy, Ph.D. – *tumor virology*
Arthur Lustig, Ph.D. – *regulation of telomere*
Asim Abdel-Mageed, D.V.M., Ph.D. – *therapeutic resistance in prostate cancer*
Charles Miller, Ph.D. – *environmental carcinogenesis*
Augusto Ochoa, M.D. – *tumor-induced tolerance*
Radhika Pochampally, Ph.D. – *cancer stem cells*
Madhwa Raj, Ph.D. – *vitamin binding proteins*
Alistair Ramsay, Ph.D. – *tumor vaccines and immune-based therapies*
Gabriele Sabbioni, Ph.D. - *cancer biomarkers*
Aline Scandurro, Ph.D. – *adult stem cells and angiogenesis*
Guoshun Wang, D.V.M., Ph.D. – *cancer viral targeting and immunotherapy*
William Wimley, Ph.D. - *protein conformation*

University of Pittsburgh

Vice Chair for Clinical Sciences Research
Department of Pharmacy and Therapeutics
School of Pharmacy

The University of Pittsburgh School of Pharmacy invites applications for vice chair for clinical sciences research, Department of Pharmacy and Therapeutics. This tenured or tenure stream position will be at the rank of associate professor or professor. The Vice Chair for Clinical Sciences Research will provide leadership for the implementation of clinical and translational sciences research and health services within the overall structure of the department and affiliated health system and guide the development and academic success of faculty within the department.

The University of Pittsburgh School of Pharmacy is one of six health science schools located on the Oakland campus. The School of Pharmacy is affiliated with the internationally renowned University of Pittsburgh Medical Center (UPMC), the region's largest and acclaimed network of tertiary, specialty, and community hospitals. Collectively, these educational, research, and clinical facilities provide one of the nation's most complete health centers for health sciences research, patient care, and teaching. The Department of Pharmacy and Therapeutics comprises 46 full-time faculty and provides pharmacy services for the UPMC health system.

The successful applicant will actively participate in the executive team of the Department of Pharmacy and Therapeutics; advance the integration of research with clinical patient care responsibilities; maintain a funded research program; and recruit and lead faculty members to improve health through excellence, innovation, and leadership in education, research, patient care, and service. The successful applicant also will contribute to the clinical pharmaceutical scientist training program within the school; play an integral role in the multi- and inter-disciplinary Clinical and Translational Sciences Institute within the University of Pittsburgh schools of the health sciences; and teach in the curricular programs of the school, including the PharmD and residency programs.

The ideal applicant will have an earned professional pharmacy degree and relevant postgraduate training; have at least five years of experience in an academic health center environment; and must document responsibilities and accomplishments in teaching, scholarship, patient care service, and leadership.

An applicant should send a complete curriculum vitae and a letter that includes a description of his or her interest in the position, a description of his or her research, and a list of the names and contact information for five individuals who will serve as references to: **University of Pittsburgh School of Pharmacy; Susan M. Meyer, PhD; Chair, Vice Chair Pharmacy and Therapeutics Search Committee; 724 Salk Hall; Pittsburgh, PA 15261;** or **smeyer@pitt.edu**.

The University of Pittsburgh is an Affirmative Action, Equal Opportunity Institution.

University of Maryland 1807 School of Medicine

Tenure Track Faculty Positions
Brain Injury and Neuroprotection Research
Department of Anesthesiology

At least two tenure-track positions at any rank are available to highly qualified individuals with outstanding records of research in the area of ischemic or traumatic brain injury. Research can involve in vivo or in vitro models of brain cell injury and death. The successful applicants will work together with a team of investigators specializing in mitochondrial dysfunction and oxidative stress. Unique opportunities exist for performing translational research in collaboration with the R Adams Cowley Shock Trauma Center. Highly competitive salary and start-up packages are available.

Applications should include a CV, a 3-page summary of research accomplishments and future plans, and the email addresses of three references to **Dr. Gary Fiskum** at **gfisk001@umaryland.edu**.

The University of Maryland is an Affirmative Action/Equal Opportunity Employer.

VANDERBILT School of Medicine

DRUG DISCOVERY AT VANDERBILT
Integrative Training in Therapeutic Discovery

A new roadmap training initiative for postdoctorals/ fellows and graduate students. Graduate students and postdoctorals/resident fellows will do research on projects to identify novel therapeutic strategies and agents. Each trainee will be co-mentored by investigators in chemical biology and translational medicine. Research training will be supplemented by coursework in chemical biology and drug discovery. Outstanding open access core resources exist in high-throughput screening, chemical synthesis, monoclonal antibody generation, natural product discovery, and small molecule NMR. Stipends, tuition, and fringe benefits are provided by a training grant from the National Institutes of Health (DA 022873).

Current collaborative projects exist in cancer, neurodegenerative and psychiatric disease, diabetes, cardiovascular disease, and infectious disease. For a complete list of projects and mentors, visit www.vanderbilt.edu/vicb/training

Applicants should contact Anne Lara, Vanderbilt Institute of Chemical Biology, (vicb@vanderbilt.edu) for additional information or to apply for the program. Please provide curriculum vitae and three letters of recommendation. Vanderbilt is an Affirmative Action/Equal Opportunity Employer.

VANDERBILT School of Medicine

Postdoctoral Research at the Center in Molecular Toxicology

Richard N. Armstrong
Michael Aschner
Nancy J. Brown
Raymond F. Burk
Richard M. Caprioli
Walter J. Chazin
David K. Cortez
Martin Egli
Brandt Eichman
F. Peter Guengerich
Tina V. Hartert
T. Alp Ikizler
Diane S. Keeney
Daniel C. Liebler
Lawrence J. Marnett
Jason D. Morrow
Jennifer A. Pietenpol
Ned A. Porter
Carmelo J. Rizzo
Michael P. Stone
William M. Valentine
Michael R. Waterman

Investigators seek new postdoctoral fellows to study various aspects of toxicology and environmental health in the Center in Molecular Toxicology through the Departments of Biochemistry, Biological Sciences, Chemistry, Medicine, Pathology, Pediatrics, and Pharmacology. Areas of investigation relating to molecular toxicology and environmental health include enzymatic oxidation and conjugation, oxidative damage, DNA damage and mutagenesis, neurotoxicology, mechanisms of cell signaling, and clinical investigations. Interested candidates should consider the laboratories of the 22 Center Investigators listed left.

For a full description of the Center and the research within the individual laboratories, please visit http://medschool.mc.vanderbilt.edu/postdoc_ad/tox/.

Applicants should submit curriculum vitae and three letters of recommendation to Dr. F. Peter Guengerich, Director, Center in Molecular Toxicology, Vanderbilt University, School of Medicine, Nashville, TN 37232-0146.

An Affirmative Action/Equal Opportunity Employer.

Skirball Institute of Biomolecular Medicine

SCHOOL OF MEDICINE
NEW YORK UNIVERSITY

Faculty Positions

The Skirball Institute and the Kimmel Center of Biology and Medicine at New York University School of Medicine invite applicants for tenure-track positions at the assistant, associate or full professor level. We seek applicants with an exceptional record of achievement to join our existing programs in Molecular Neurobiology, Developmental Genetics, Structural Biology and Molecular Pathogenesis. These programs are interdisciplinary and reflect strengths at NYU's School of Medicine and College of Arts and Sciences. Special priority will be given to applicants with broad interests working at the cutting edge of mammalian genetics, stem cell research, neurobiology or molecular cell biology.

NYU School of Medicine offers excellent resources to support new faculty, including generous start-up packages and core facilities for cell sorting, imaging, proteomics, mouse molecular genetics, genomics and structural biology.

Successful candidates are expected to initiate and maintain vigorous independent research programs that will enrich and be enriched by the highly collaborative environment at the Skirball Institute and throughout the NYU research community.

This is an electronic application process. No mail applications will be accepted. Create your application packet by formatting it as a single PDF document. Use the following page order: (1) Cover Letter, (2) Curriculum Vitae, (3) Research Statement.
Email application packet to **skirballsearch@saturn.med.nyu.edu**.

Three letters of reference should be sent independently to: **skirballsearch@saturn.med.nyu.edu**

New York University School of Medicine was founded in 1841 and is an equal opportunity affirmative action employer. Women and minority candidates are encouraged to apply.

http://saturn.med.nyu.edu

Rat der Eidgenössischen Technischen Hochschulen ETH-Rat | Conseil des écoles polytechniques fédérales CEPF | Consiglio dei politecnici federali CPF | Cussegl da las scolas politecnicas federalas CSPF | Board of the Swiss Federal Institutes of Technology ETH Board

The ETH Board is pleased to announce a challenging opportunity for the right cosmopolitan and creative personality:

President
The Swiss Federal Institute of Technology in Zurich (ETH Zurich)

The ETH Zurich is a leading science and technology university with world-renowned researchers and teachers. The university earned its distinguished national and international reputation through both major achievements in research as well as first-rate teaching and services. 18'000 people from Switzerland and abroad are currently studying, working or conducting research at ETH Zurich. Superior research conditions, state-of-the-art infrastructure and an attractive urban environment create the ideal conditions for creative personalities here to flourish.

Requirements
Candidates must demonstrate an open-minded, decisive, creative, and broadly interested personality, along with an excellent track record over the course of his or her career. The successful candidate has proven scientific, technological and social leadership skills, as well as excellent management and interpersonal credentials from international academia and/or private business and industry. Comprehension of the specifics of university management is a prerequisite. The president of ETH Zurich must be prepared to take autonomous decisions, while accepting the necessity for active participation in practical issues involving all aspects of the institution; this will entail the direction of a functional but non-hierarchical management culture. He or she must furthermore have excellent communication skills in relation to both the public and the academic community, with perfect knowledge of German and English, and good comprehension of French. Knowledge of European and Swiss political and legislative practice is an advantage.

Starting date of presidency: September 1, 2007

Please send your written application by January 31, 2007, to the attention of Prof. Alexander Zehnder, President of the ETH Board, ETH Zentrum, CH – 8092 Zurich.

The Medical College of Georgia Vascular Biology Center is recruiting a **pulmonary vascular biologist at the Assistant, Associate or Full Professor level**, tenure-track. The successful candidate will have an earned Ph.D., M.D. or M.D./Ph.D. degree. He/she will join an active group of extramurally funded vascular biologists (currently about $8 million annually, see: **http://www.mcg.edu/centers/VBC/index.html**) in recently renovated laboratories utilizing state-of-the-art equipment. He/she will have the opportunity to participate in the two institutional pre- and post-doctoral training programs in Integrative Cardiovascular Biology. Ample opportunities for collaborative basic and clinical research are available and encouraged. The candidate is expected to have and further develop an active, extramurally funded research program in aspects of pulmonary vascular disease, especially acute lung injury. Highly competitive salary and start-up package, commensurate with prior experience, will be provided.

Applications should include detailed CV, statement of career goals and names of three references and e-mailed to **John D. Catravas, Ph.D. (jcatrava@mcg.edu)**.

The Medical College of Georgia is an AA/EOE. Applications from women and under-represented minorities are particularly encouraged.

ASSISTANT/ASSOCIATE PROFESSOR in PROTEIN STRUCTURE AND FUNCTION

The **Department of Biochemistry, in collaboration with the Department of Physiology,** seeks outstanding candidates for a tenure-track faculty position at the rank of Assistant/Associate Professor to complement existing strengths in structural biology and biophysics. We are particularly interested in candidates **using biophysical methods** (e.g. NMR, X-ray crystallography, cryo-EM, etc.) **to probe the structure and action of membrane proteins.** The successful applicant is expected to develop a vigorous, extramurally funded research program, and contribute to the graduate teaching mission of the HSC. The Biochemistry Department is undergoing expansion through recruitment of additional junior positions and an ongoing search for a second Robert A. Welch Foundation Distinguished Professor of Chemistry. There are 22 primary and 8 cross-appointed faculty members in the Department, which also houses state-of-the-art instrumentation in University Core Facilities for NMR, Analytical Ultracentrifugation, Mass Spectrometry, Surface Plasmon Resonance, and X-ray Crystallography. Details about the Department faculty and its Core facilities can be found at **http://www.biochem.uthscsa.edu**. The campus of the HSC is located ~20 minutes from the center of multi-cultural San Antonio, and at the gateway to the Hill Country and its wealth of outdoor and cultural activities.

Applicants should send a cover letter, current curriculum vitae and a 2-3 page description of future research plans to **pjhart@biochem.uthscsa.edu**. Three letters of support from individuals familiar with the applicant's potential should be sent or emailed to: **P. John Hart, Ph.D., Ewing Halsell/President's Council Distinguished Chair, Department of Biochemistry, MSC 7760, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, FAX: 210-567-6595**. All faculty appointments are designated as security sensitive positions.

The University of Texas Health Science Center at San Antonio is an Equal Employment Opportunity/Affirmative Action Employer.

PSORIASIS IMMUNOGENETICS
Assistant Professor

Assistant Professor (tenure-track) in psoriasis immunogenetics clinical research. The position requires doing psoriasis research involving cellular immunology, human genetic linkage and association analysis, and microarray analysis of gene expression and genetic variation. This research requires performance of biopsies of human skin by punch and keratome techniques and providing research related clinical care. Qualifications: M.D. and post-doctoral training in immunology and human genetic linkage and association analysis; Ph.D. degree involving genomics, immunology and human genetic linkage and association, desirable; and related research publication record in genetics and immunology focused on psoriasis.

Send CV and references to:
John J. Voorhees, MD FRCP
University of Michigan
Dept of Dermatology
1910 Taubman Ctr
Ann Arbor, MI 48109-0314

University of Michigan
Medical School

An Equal Opportunity / Affirmative Action Employer.

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Tenure Track Assistant Professor Position in Sustainable Energy

at Ecole Polytechnique Fédérale de Lausanne (EPFL)

EPFL is planning a substantial expansion of its School of Architecture, Civil and Environmental Engineering.

In order to develop research and educational programs in the field of the renewable energies and their integration within various infrastructures (energy production and transportation facilities, energy distribution networks, municipal infrastructures as well as industrial, commercial and residential buildings), we are looking for a **Tenure Track Assistant Professor** in sustainable energy.

A strong expertise in the renewable energy science and technologies is desired. Research areas of interest include wind energy, biofuels, hydroelectric, solar thermal, geothermal and fuel-cells as well as various energy storage technologies. The successful candidate is expected to initiate independent, creative research programs and participate in undergraduate and graduate teaching.

Significant start-up resources and research infrastructure will be available. Internationally competitive salaries and benefits are offered.

To apply, please follow the application procedure at http://enac.epfl.ch/facultypositions. The following documents are requested in PDF format: motivation letter, curriculum vitae, publications list, concise statement of research and teaching interests, as well as the names and addresses (including e-mail) of at least five referees. Screening will start on **April 15th, 2007.** Further questions can be addressed to :

Professor Laurent Vulliet, Dean
School of Architecture, Civil and Environmental Engineering, EPFL
CH-1015 Lausanne
Switzerland

For additional information on EPFL, please consult:
http://www.epfl.ch or http://enac.epfl.ch

EPFL is an equal opportunity employer

NIEHS
National Institute of Environmental Health Sciences

Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

Editor-in-Chief

The National Institute of Environmental Health Sciences is commencing a search for the next Editor-in-Chief of *Environmental Health Perspectives (EHP)*. *EHP* is a peer-reviewed monthly science journal, publishing a wide range of topics related to the impact of the environment on health and disease. The journal has an impact factor of 5.34 and ranks first among 132 environmental science journals and among 90 public, environmental, and occupational health journals. The journal is international in scope and is distributed in 190 countries. The Editorial Search Committee seeks to identify an active scientist in a field related to the environmental health sciences and with previous editorial experience. The objective is to identify the next Editor-in-Chief by February 1, 2007. This individual will then begin working with the Interim Editor and *EHP* staff to complete the transition by July 1, 2007.

Letters of interest and plans for *EHP*, along with curriculum vitae, should be submitted by **December 1, 2006** either electronically or by mail to:

William J. Martin II, M.D.
National Institute of Environmental Health Sciences
PO Box 12233, Mail Drop B2-07
Research Triangle Park, NC 27709
E-mail: lloyd3@niehs.nih.gov

DHHS and NIH are Equal Opportunity Employers.

WEILL CORNELL
MEDICAL COLLEGE IN QATAR

FACULTY POSITIONS

In a pioneering international initiative, Weill Medical College of Cornell University established the Weill Cornell Medical College in Qatar (WCMC-Q) with the sponsorship of the Qatar Foundation for Education, Science and Community Development. WCMC-Q is located in Doha, Qatar, and in its fifth year of operation, Weill Cornell seeks candidates for faculty positions to teach in Doha in:

NEUROPHYSIOLOGY

Following a two-year Pre-medical Program, the inaugural class has now completed the second year of the traditional four-year education program leading to the Cornell University M.D. degree, which they will receive in May 2008. The medical program at WCMC-Q replicates the admission standards and the innovative problem-based curriculum, which includes, among other things, integrated, multidisciplinary basic science courses that are the hallmark of the Weill Medical College of Cornell University.

Faculty, based in Doha, will be expected to teach their specialty and to contribute to the academic life of the Medical College. This unique program provides the successful applicant with the opportunity to leave his/her mark on a pioneering venture. A state of the art research program, to be housed in WCMC-Q and focused on genetics and molecular medicine and women and children's health will be initiated within the next year. Teaching and research facilities are situated within a brand new building designed to Cornell specifications and located in Education City in Doha amongst other American universities.

All faculty members at WCMC-Q are appointed by the academic departments at Weill Cornell.

Further details regarding the WCMC-Q program and facilities can be accessed at:

www.qatar-med.cornell.edu.

Candidates should have a M.D., Ph.D. or M.D./Ph.D. or equivalent terminal degree. The successful candidate will have strong teaching credentials and experience in teaching medical students. Salary is commensurate with training and experience and is accompanied by an attractive foreign-service benefits package.

Qualified applicants should submit a letter of interest outlining their teaching and research experience and curriculum vitae to:

facultyrecruit@qatar-med.cornell.edu

***Please quote Faculty Search #06-009-sci on all correspondence**

Cornell University is an equal opportunity, affirmative action educator and employer.

The screening of applications will begin immediately and continue until suitable candidates are identified.

Please note, due to the high volume of applications, only short-listed candidates will be contacted.

UNIVERSITY OF MARYLAND

THE DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

Bio-molecular NMR Spectroscopist

All Levels Tenure-track or tenured position (Assistant Professor, Associate Professor, and Professor)

As part of major University initiatives in the life sciences and biophysics, the Department of Chemistry and Biochemistry seeks to appoint a bio-molecular NMR spectroscopist to a tenured or tenure-track position. The successful candidate will add to the existing programs in biomolecular structure and organization and molecular biophysics. The focus of this search is on individuals who have strong interest in NMR methodology and applications to biomolecular structure and function. We seek outstanding scientists whose research interests complement existing strengths in the department and across the University and who are committed to developing outstanding programs in research and teaching. One of four departments within the College of Chemical and Life Sciences, members of the Department of Chemistry and Biochemistry participate in university centers including the Center for Biomolecular Structure and Organization, the Center for Bioinformatics and Computational Biology, the Institute for Physical Science and Technology, as well as university-wide initiatives in biophysics. The University of Maryland, College Park is the flagship campus of the University of Maryland System and is ideally situated in close proximity to Washington, D. C., Baltimore, and Maryland's 270 Technology Corridor. Candidates should submit via department website (**www.chem.umd.edu/employment.html**) a curriculum vitae, a three-page summary of research plans, a statement of educational interests, and contact information for three persons from whom letters of recommendation may be requested.

Qualifications: We seek scholars who will build or have highly visible, widely acclaimed research programs and who are capable of excellence in undergraduate and graduate education. Candidates are expected to have a Ph.D. degree, demonstrated accomplishments in independent research, and promise as an effective educator.

Salary: Commensurate with qualifications.

Deadline: Review of applications will begin **February 15, 2007**, but we will continue to accept applications until the position is filled. *EOE/AA.*

APPLICATIONS FROM WOMEN AND MINORITIES ARE ENCOURAGED.

MIT Nuclear Science & Engineering

Assistant Professor

The MIT Nuclear Science and Engineering Department invites applications for a tenure track position at the Assistant Professor level in the broad area of nuclear science and engineering, including fission energy, radiation science, and plasma physics and fusion technology. While the department is particularly seeking to strengthen the areas of fission reactor physics and nuclear chemical engineering, outstanding candidates with expertise in disciplines across the entire field of nuclear science and engineering will be given serious consideration. Applicants should have a Ph.D. in an engineering or physical sciences discipline and demonstrated excellence in research. Responsibilities will include teaching undergraduate and graduate subjects in nuclear science and engineering.

To apply, submit a curriculum vitae, description of research interests, and the names of three references via e-mail to nsefacultysearch@mit.edu or by mail to MIT, Nuclear Science and Engineering Dept, Faculty Search, Room 24-124, 77 Massachusetts Avenue, Cambridge, MA 02139-4307.

MIT is an equal opportunity/affirmative action employer. Applications from women, minorities, veterans, older workers, and individuals with disabilities are strongly encouraged.

http://web.mit.edu

Tenure-Track Faculty Positions in Structural Biology

Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center invites applications for a tenure-track faculty position at the Assistant Member level in the Structural Biology Program of the Sloan-Kettering Institute (www.ski.edu). We are interested in individuals with an outstanding record of research achievements in any area of structural biology, including x-ray crystallography, NMR spectroscopy, EM and optical imaging, as well as the interface of structural, chemical and computational biology. Faculty will be eligible to hold appointments in the newly established Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, as well as the Weill Graduate School of Medical Sciences of Cornell University.

Interested individuals should submit their Curriculum Vitae, description of past research accomplishments and proposed research, selected reprints and three letters of recommendation to: **strucbio@mskcc.org.** Application materials can also be submitted to: **Dr. Nikola Pavletich, c/o Tiffany Lennon, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 135, New York, New York 10021.** The application deadline is March 15, 2007. Memorial Sloan-Kettering Cancer Center is an Equal Opportunity Employer.

Memorial Sloan-Kettering Cancer Center

The Best Cancer Care. Anywhere.

www.mskcc.org

universitätbonn

Applications are being accepted for the position of: Full Professor (W3, tenure) for Crop Science

The professorship will be part of the Institute of Crop Science and Resource Conservation (INRES) in the Faculty of Agriculture, University of Bonn, Germany.

The holder of the position will be responsible for teaching undergraduate and graduate level courses in Agronomy and Crop Science in the study programmes "Agricultural Sciences", "Nutrition and Household Sciences" and in "Agricultural Science and Resource Management in the Tropics and Subtropics" (ARTS). The ability to teach in English is necessary for specific advanced courses.

Applicants should be highly qualified to teach and conduct research in at least one of the following research areas: crop physiology including yield physiology, precision farming or crop modelling.

Interdisciplinary cooperation in both research and teaching with the departments of the Faculty of Agriculture as well as with the Faculty of Mathematics and Natural Sciences is expected. International recognition within the scientific community as well as research experience in and outside Europe is considered relevant.

Applications must comply with section 46 of University law (§ 46 - as from January 1, 2007 § 36 - HG NRW).

Please send your application including a CV, a list of publications and a statement of teaching and research interests to: **Dean, Faculty of Agriculture, University of Bonn, Meckenheimer Allee 174, D - 53115 Bonn, Germany.** The deadline for application is **March, 15, 2007**.

The University of Bonn is committed to enhancing the diversity of its faculty and staff and especially encourages applications from women and people with disabilities. In cases of equal qualification, preference will be given to women or persons with disabilities.

Faculty Position in Marine Ecology/Conservation Biology
Scripps Institution of Oceanography
University of California, San Diego

The Scripps Institution of Oceanography (SIO) **http://sio.ucsd.edu/** at the University of California, San Diego (UCSD) invites applications for an Assistant Professor (tenure track) in the broadly defined area of marine ecology and conservation biology. We are particularly interested in applicants able to cross disciplinary boundaries and work with a diverse array of colleagues including physical and social scientists. Applicants must hold a Ph.D. degree or equivalent and postdoctoral experience is preferred. The successful applicant will be expected to conduct and fund an active research program, advise graduate students, and teach at both the undergraduate and graduate levels. Applicants are expected to show evidence of their potential by a publication record appropriate for their experience. The salary will depend on the experience of the successful applicant and will be based on the University of California pay scale.

Review of applications will begin on **February 15, 2007** and will continue until position is filled. Applicants should submit their CV, a letter including descriptions of research interests and teaching interests/experience, a list of publications, immigration status, and the names of three people with their contact information, complete institution address, email address, phone and fax nos., who are familiar with the applicant's research to: **Chair, SIO Graduate Department, 0208, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0208, USA**. ***Please clearly label applications "Marine Ecology/Conservation Biology Search"***. Applicants are welcome to include in their cover letters a personal statement summarizing their contributions to diversity.

UCSD is an Equal Opportunity Employer
with a strong institutional commitment to excellence through diversity.

FACULTY POSITION IN APPLIED PHYSICS

The Applied Physics Program at Caltech invites applications for one tenure track position as assistant professor. We are seeking highly qualified candidates who are committed to a career in research and teaching. Exceptionally well-qualified candidates may be considered at the associate or full professor level. In addition to applicants from traditional areas including device and/or materials physics we are interested in applicants with interdisciplinary backgrounds spanning these and other areas such as biology and chemistry.

Interested applicants should submit an electronic application by visiting **http://www.eas.caltech.edu/search/aph**. You will be asked to upload the following pdf documents: CV, research statement, three publications, and the names and contact information for three references.

The term of the initial appointment is normally four years, and appointment is contingent upon completion of all the requirements for a Ph.D.

CALIFORNIA INSTITUTE OF TECHNOLOGY
Division of Engineering and Applied Science
Caltech is an Equal-Opportunity/Affirmative-Action Employer.
Women, minorities, veterans, and disabled persons are encouraged to apply.

www.cam.ac.uk/jobs/

University Non-Clinical Lectureships in Reproductive Biology (Two posts)

Department of Obstetrics & Gynaecology

£32,471 to £41,133 pa

Applications are invited from candidates for two UFC funded lectureships in Reproductive Biology. The primary responsibility of the posts is to lead a programme of research in an area of Reproductive Biology. Candidates with an interest in any area relating to Women's Health are potentially appointable (including gynaecological cancer and fetal physiology): the primary requirement is outstanding potential as an independent scientist. However, the department has current programmes, which utilise gene array and transgenic mice and focus on placental biology and angiogenesis. Candidates with expertise in these areas would be particularly welcome. The people appointed should have a PhD, post-doctoral research experience and a proven track record of publications and successful grant applications. The appointment will be for 5 years in the first instance with re-appointment until retirement age with appropriate performance.

For an information pack email Ms L Foster, LMDF2@medschl.cam.ac.uk. Closing date for applications (10 copies), together with a curriculum vitae and the names of three referees, is 31 January 2007.

UNIVERSITY OF CAMBRIDGE

The University offers a range of benefits including attractive pension schemes, professional development, family friendly policies, health and welfare provision, and staff discounts. The University is committed to equality of opportunity.

FACULTY POSITION IN BIOENGINEERING

The **California Institute of Technology** invites applications for one tenure-track faculty position in the general area of biological engineering. The specific research area of the candidate is not as critical as is the ability to integrate engineering and biological principles into the synthesis process. Relevant research interests include, but are not limited to, multi-cellular and tissue level engineering, novel methods to synthesize biological material, and biological interfaces. Also relevant to our search is fundamental research aimed at developing greater understanding of key mechanisms that will underlie future bioengineering synthesis activities. Researchers whose techniques span the molecular, cellular, and tissue scales are particularly encouraged to apply.

We seek highly qualified candidates committed to a career in research and teaching. Strong preference will be given to applicants at the assistant-professor level; however, full consideration will be given to more senior applicants with a distinguished research record. Initial appointments at the assistant-professor level are for four years, and are contingent on completion of the Ph.D. degree.

The Bioengineering Option is a joint program of the Divisions of Engineering and Applied Science, Chemistry and Chemical Engineering, and Biology. The successful candidate will have a primary appointment in Engineering and Applied Science. Joint appointments in other divisions can be arranged as appropriate. Further information about our Bioengineering program can be found at **http://www.be.caltech.edu.**

Qualified candidates should submit a letter of application (pdf format) consisting of a curriculum vitae, a statement of research interests, a statement of teaching interests, the names and contact information for at least four references, and up to three representative publications to: **http://www.eas.caltech.edu/search/bioengineering**. Review of the applications will commence on January 15, 2007 and continue until the position is filled.

CALIFORNIA INSTITUTE OF TECHNOLOGY
Division of Engineering and Applied Science
Caltech is an Equal-Opportunity/Affirmative-Action Employer.
Women, minorities, veterans, and disabled persons are encouraged to apply.

University of Pittsburgh
Faculty Positions
Center for Vaccine Research

The Center for Vaccine Research (CVR) of the University of Pittsburgh is seeking outstanding scientists for several tenure and tenure-track positions at the Assistant, Associate, or Professor levels. Appointments are available in the School of Medicine, the Graduate School of Public Health, or one of the other schools of the health sciences.

The CVR is housed in the new, state-of-the-art, 300,000 sq. ft. Biomedical Research Tower-3 (BST3), which is located on the main campus of the University of Pittsburgh—one of the nation's leading research institutions. The CVR is composed of two components—the Vaccine Research Lab (VRL) and the Regional Biocontainment Lab (RBL). The VRL occupies 12,000 sq. ft. of laboratory and office space, including dedicated BSL2+ tissue culture rooms, instrument rooms, and office and conference rooms. Located adjacent to several related units (e.g., Genomics and Proteomics Core Labs and Drug Discovery Institute), the VRL offers an outstanding interactive research environment by providing access to microarray, robotic, and mass spectrometry instrumentation. The RBL is currently under construction and is expected to open in mid-2007. It will occupy 20,000 sq. ft. and offer comprehensive BSL3 lab space and ABSL3 facilities (small animals and nonhuman primates) which will include an aerosol challenge module as well as surgical, necropsy, and imaging suites.

Qualifications include academic accomplishments that meet the standards for a tenure-track appointment, including an advanced degree (M.D., Ph.D., or equivalent). Successful candidates will have a sound publication record, be active contributors to the vaccine research field, and have a demonstrated ability to obtain extramural research funding. Candidates should be prepared to establish an independent laboratory program in vaccine research. Individuals with expertise in immunology of infectious diseases, microbial pathogenesis, or microbial vaccines are encouraged, as are those with a translational component to their research. Salary, rank, and academic appointment will be commensurate with qualifications and experience.

Review of applications will begin immediately and continue until all positions are filled. Interested individuals should submit a letter of application, curriculum vitae, a statement of research accomplishments and goals, and the names, mailing addresses, e-mail addresses and telephone numbers of three professional references. Electronic applications are preferred and should be sent to **CVRInfo@pitt.edu (subject line: CVR Faculty Search)**. Applications submitted by mail should be sent to:

University of Pittsburgh
CVR Search Committee c/o Donald S. Burke, MD
Director, Center for Vaccine Research, University of Pittsburgh
9014 Biomedical Science Tower 3, 3501 Fifth Avenue, Pittsburgh, PA 15261, USA
Inquiries: e-mail CVRInfo@pitt.edu or telephone 412-624-3001
The University of Pittsburgh is an equal opportunity, affirmative action employer.
Women and minority candidates are strongly encouraged to apply.

GEORGETOWN UNIVERSITY

The **Lombardi Comprehensive Cancer Center (LCCC)** at **Georgetown University** invites applications and nominations for the position of **Director, Lombardi Comprehensive Cancer Center**. The Director will bring dynamic scientific, clinical and business leadership to the Cancer Center. This role represents an exceptional opportunity to shape and lead a program that engages a team of clinicians and scientists who will conduct cancer-related research and provide state-of-the-art patient care. The Director of the Lombardi Comprehensive Cancer Center reports to the Executive Vice President for Health Sciences and Executive Dean of the School of Medicine. The Director of the LCCC is responsible for advancing the basic, population services and clinical research, clinical care, and community outreach that are its mission, working effectively with the leadership of MedStar to exceed its own goals for quality delivery of patient care.

The Director will combine a compelling vision for the training and research of basic and clinical biomedical science, leveraging interdisciplinary collaborations to: integrate discovery activity; increase the rate of interventions development and the application of innovative technologies through translational research; and secure delivery of these interventions for use in the clinic and in public health initiatives as progressive care for cancer patients. The Director will be responsible for leading the Center into the top tier of comprehensive cancer programs in the world. Lombardi seeks a leader who can assess its assets, recombining them to capitalize on current strengths and create a strategy of near- and long-term targets for clinical, translational and basic research opportunities.

We are seeking an M.D. and/or M.D./Ph.D. in the biomedical sciences with a proven record of successful administration of a cancer center, a related department or biomedical research organization. The Director will have a broad reputation for excellence in research, education and/or healthcare delivery, which affords the Director credibility and stature within the field.

Russell Reynolds Associates is working closely with a Search Committee to facilitate this important search. Please forward materials to: **Anne Martin Simonds, Russell Reynolds Associates, 1701 Pennsylvania Avenue NW, Suite 400, Washington, DC 20006; jrackley@russellreynolds.com.**

INDIANA UNIVERSITY SCHOOL OF MEDICINE

Asthma and Allergic Disease Program
Assistant/Associate Professor

The Department of Pediatrics, Section of Pulmonology, Critical Care and Allergy and the HB Wells Center for Pediatric Research (www.wellscenter.iupui.edu) is recruiting two faculty positions at the Assistant/Associate Professor level. We are particularly interested in candidates working in asthma and allergic disease related research to complement existing strengths in cytokine and T cell biology, and airway physiology. Candidates will have a PhD, MD or MD/PhD and must have a strong research background and either current, or potential for, independent funding. New faculty will be provided with generous start-up packages and join an active multi-disciplinary Immunology and Airway Disease research community that was recently funded by an Asthma and Allergic Diseases Research Center.

Interested candidates are encouraged to submit a curriculum vitae and a short description of research interests to: **Mark H. Kaplan, PhD, Director of Pediatric Pulmonary Basic Research, Wells Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, 702 Barnhill Drive, Room 2612, Indianapolis, IN 46202, airway@iupui.edu**.

Indiana University is an EEO/AA Educator, Employer and Contractor (M/F/D).

Tenure Track Faculty Positions
Viral Pathogenesis-Inflammation

Fox Chase Cancer Center is seeking to recruit new tenure track faculty at the level of Associate Member (equivalent to Assistant Professor) or Member (equivalent to Associate Professor) to join the Viral Pathogenesis program.

We are particularly interested in candidates that will use in vivo models of viral infection to investigate mechanisms of one or several of the following: innate immunity, adaptive immunity, tolerance, inflammation or carcinogenesis.

The successful candidates must have outstanding credentials and will be expected to establish a strong, extramurally funded research program. Fox Chase Cancer Center is an independent research institution that offers a generous start up package and a highly interactive environment.

Application package must be submitted electronically to **Skalka.Recruitment@fccc.edu.** Package should include curriculum vitae, a concise research statement describing research accomplishments and future research plans (4 pages maximum) and contact information for at least three referees. Please reference "Viral Pathogenesis faculty position" in your cover letter.

FOX CHASE
CANCER CENTER

Fox Chase Cancer Center is an Affirmative Action/ Equal Opportunity Employer and solicits applications from women and under-represented minorities.

BioPAD

LIFE*lab*

iThemba Pharmaceuticals (Pty) Ltd
Johannesburg, South Africa

Funded by LIFE*lab* and BioPAD, South African based iThemba Pharmaceuticals aims to become a world-class, research-based, bio-pharmaceutical company. This private company was founded by leading medicinal chemists, **Dennis Liotta** (Emory Univesity USA), **Steven Ley** (Cambridge University, UK), **Anthony Barrett** (Imperial College, UK) and **George Painter** (Chimerix Inc, USA) to discover and develop medicines for the healthcare needs of Africa.

Chief Scientific Officer and/or Director of Chemistry

iThemba Pharmaceuticals (Pty) Ltd, is seeking to recruit a Chief Scientific Officer (CSO) and/or Director of Chemistry. The incumbent will work with a team of internationally renowned medicinal chemists in the field of infectious diseases and should have extensive medicinal chemistry experience within the pharmaceutical industry, as well as strong management skills. The CSO will provide both the scientific and business leadership to iThemba Pharmaceuticals during and after the start up stage of the company.

The key requirements for the CSO/Director of Chemistry position are:

- A PhD in synthetic organic chemistry. The candidate must be an internationally renowned industrial scientist with a minimum of 5 years experience of drug discovery and development in the pharmaceutical and/or biotech industries
- Knowledge and understanding of: local (South African) and international pharmaceutical/biotechnology sector; principles of financial management and the use of appropriate management tools; cost and budgetary control
- Proven ability to negotiate and close contracts
- Motivated, committed, innovative and decisive individual with vision, strong marketing, networking and strategic thinking skills who thrives in a dynamic, entrepreneurial environment and is willing to travel

A highly incentivised, competitive package, which reflects experience and skills, is offered for an initial two year contract position. **Applications:** Kindly forward a letter of motivation together with your CV to **Dr. Carl Montague** at **carl.montague@lifelab.org.za** by the **28 January 2007**. For further information please contact: **Dr. Agatha Masemola (agatha@biopad.org.za)** or **Prof. Dennis Liotta (dliotta@emory.edu)**.

WEILL CORNELL
MEDICAL COLLEGE IN QATAR

FACULTY POSITIONS

In a pioneering international initiative, Weill Medical College of Cornell University established the Weill Cornell Medical College in Qatar (WCMC-Q) with the sponsorship of the Qatar Foundation for Education, Science and Community Development. WCMC-Q is located in Doha, Qatar, and in its fifth year of operation, Weill Cornell seeks candidates for faculty positions to teach in Doha in:

BIOCHEMISTRY

Following a two-year Pre-medical Program, the inaugural class has now completed the second year of the traditional four-year education program leading to the Cornell University M.D. degree, which they will receive in May 2008. The medical program at WCMC-Q replicates the admission standards and the innovative problem-based curriculum, which includes, among other things, integrated, multidisciplinary basic science courses that are the hallmark of the Weill Medical College of Cornell University.

Faculty, based in Doha, will be expected to teach their specialty and to contribute to the academic life of the Medical College. Biochemistry is taught at the pre-medical level and as a component of the Molecules, Genes and Cells course in the medical curriculum. Candidates must be able to teach the biochemistry of metabolism, in addition to molecular biology. This unique program provides the successful applicant with the opportunity to leave his/her mark on a pioneering venture. A state of the art research program, to be housed in WCMC-Q and focused on genetics and molecular medicine and women and children's health will be initiated within the next year. Teaching and research facilities are situated within a brand new building designed to Cornell specifications and located in Education City in Doha amongst other American universities.

All faculty members at WCMC-Q are appointed by the academic departments at Weill Cornell.

Further details regarding the WCMC-Q program and facilities can be accessed at:

www.qatar-med.cornell.edu.

Candidates should have a M.D., Ph.D. or M.D./Ph.D. or equivalent terminal degree. The successful candidate will have strong teaching credentials and experience in teaching medical students. Salary is commensurate with training and experience and is accompanied by an attractive foreign-service benefits package.

Qualified applicants should submit a letter of interest outlining their teaching and research experience and curriculum vitae to:

facultyrecruit@qatar-med.cornell.edu

***Please quote Faculty Search #06-008-sci on all correspondence**

Cornell University is an equal opportunity, affirmative action educator and employer.

The screening of applications will begin immediately and continue until suitable candidates are identified.

Please note, due to the high volume of applications, only short-listed candidates will be contacted.

University of Heidelberg

The **Ruprecht-Karls-Universität** in Heidelberg invites applications for the position of

Rector.

The University of Heidelberg is the oldest university in Germany, with six centuries of tradition in teaching and research. Its ethos is that of a classical university with a broad range of subjects and a strong emphasis on research. Interdisciplinary cooperation is an essential element of its academic achievement, at the national and international level. The University of Heidelberg has a budget of approx. € 168 million (excluding Medicine), a good proportion of third-party funding, about 27,000 students from all subject areas, just under 400 full professors, a total of over 11,000 staff and extensive land and property.

Heidelberg University is looking for an eminent academic with strong leadership qualities. He/she should have special experience in the management of large organisations and a well-developed understanding of what is involved in running a university. In coordination with the University Council and the internal self-governance structures, applicants should be able to demonstrate a policy of innovation and integration in guiding the university through the current processes of change in all areas – from the "minor" disciplines to the major field of university medicine. The Rector will be expected to advance cooperation with top-level research institutions in the Heidelberg area, and to pursue a forward-looking policy in expanding the network of international cooperations.

The appointment will commence on October 1, 2007. The Rector represents the University; responsibilities, powers, preconditions for appointment and service ranking follow from §17 of the higher-education law of Baden-Württemberg. The Rector is a public servant with a fixed-term contract; the term of office is six years and re-election is possible. The successful candidate will be elected by the University Council, the decision will be ratified by the University Senate and the state premier of Baden-Württemberg will make the final appointment.

Heidelberg University is anxious to increase the share of women on its staff and so strongly encourages qualified women to apply. Candidates with disability will be given preference, all other things being equal.

Applications with all appropriate documents should be sent by **26 January 2007** to the **Chairman of the University Council of Heidelberg University, Office of the University Council, Seminarstraße 2, 69117 Heidelberg, Germany.**

Training in Infectious Disease Research

University of Pennsylvania
School of Veterinary Medicine
Veterinary Center for Infectious Diseases

As part of a new initiative to expand infectious disease research at the School of Veterinary Medicine, the Veterinary Center for Infectious Diseases invites applications for **RESEARCH FELLOWSHIPS** from veterinarians aiming for a career in which research is a principal component. Candidates must have a veterinary degree and evidence of research potential. Board certification in any relevant clinical specialty is desirable but not essential. Successful candidates will initiate basic or translational research projects under the mentorship of established investigators at the School.

Fellowships will initially be for one year, with the potential for a second year depending on progress and available funding. Applicants should submit a letter of intent, CV, contact information and the names and contact information for three referees to: **Ms. Louisa Dowling, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce St., Philadelphia, PA 19104-6008; Telephone: 215-898-7898**; E-mail: **dowling@vet.upenn.edu**. The deadline for applications is **April 1, 2007**. Specific questions about the fellowships may be directed to **Dr. Edward Pearce; Telephone: 215-573-3493**; E-mail: **ejpearce@mail.med.upenn.edu**.

The University of Pennsylvania is an Affirmative Action/Equal Opportunity Employer.

UNIVERSITY OF MARYLAND
Baltimore

Dentistry / Law / Medicine / Nursing / Pharmacy / Social Work / Graduate Studies / Public Health

Director, Stem Cell Initiative
Associate Professor or Full Professor
School of Medicine

Applications are invited from established, highly productive, dynamic individuals with exceptional expertise in the area of basic stem cell biology. We seek an individual with a creative vision, to establish and lead a new stem cell initiative at the School of Medicine, University of Maryland, Baltimore. The School of Medicine has an aggressive research vision and offers a vibrant interdisciplinary research environment. The School is on a rapid upward trajectory, and currently ranks among the top 25 medical schools in the country in NIH funding.

This accomplished individual would take the leadership role in catalyzing a major stem cell research program at University of Maryland School of Medicine, uniting growing areas of expertise in neuroscience, hematopoetic and cardiovascular stem cell research.

The successful candidate is expected to have a superb record of scholarly activity, a continuous history of extramural funding together with proven leadership, and administrative abilities, experience building successful interdisciplinary programs and a commitment to further expand the high national and international visibility of the School.

Nominations and applications should be sent to: Dr. Meredith Bond, Chair, Search Committee for Director, Stem Cell Initiative, at stemcellsearch@som.umaryland.edu. Applicants should submit, by email, a letter summarizing their qualifications and interest in the position, with an updated CV. The letter should describe research, teaching, service, administrative experience, previous mentoring, and achievements with interdisciplinary programs. All inquiries, nominations and applications will be treated confidentially.

For more information, please visit the University of Maryland School of Medicine website at http://medschool.umaryland.edu. For questions or additional information please contact Dr. Bond, mbond@som.umaryland.edu. Review of applications will begin March 1, 2007.

University of Maryland is an Equal Opportunity/ Affirmative Action Employer.

www.hr.umaryland.edu

Donald Danforth Plant Science Center

DISCOVER • ENLIGHTEN • SHARE • NOURISH

Director and PI Positions
Institute for Renewable Fuels

The Donald Danforth Plant Science Center seeks a Director and an additional principal investigator at either the Assistant or Associate Member level for the newly established Institute for Renewable Fuels. The Institute will focus on enhancing production and recovery of energy-rich biofuels components and co-products from plant materials. The Director will coordinate and lead the efforts of the Institute and maintain a vigorous research program in the biofuels area. Research projects will support the focus of the Institute and complement current strengths in plant biochemistry and metabolic engineering at the Danforth Center (**www.danforthcenter.org**). Preference will be given to applicants with multidisciplinary research programs, ongoing research support, and proof of scientific productivity. Level of appointment and salary will reflect prior experience.

Applicants should submit cv, reprints of three significant publications, and description of future research to: **Ms. Billie Broeker, Director of Human Resources, REF: Plants, Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO, 63132**, or by email to **bcbroeker@danforthcenter.org**, with Plants in the subject line.

EOE/AA Employer/M/F/D/V.

Post-Doctoral Fellows

We have immediate openings for qualified and highly motivated researchers to pursue post-doctoral training. GIS provides a rich academic environment for post-docs to engage in research that applies cutting-edge technologies in genomics, genetics, proteomics, and bioinformatics to address questions in the biology of stem cells, cancer, and immunity. Post-doctoral fellows receive internationally competitive funding and travel allowances to attend scientific conferences. Current openings are described below. Please visit our website **www.gis.a-star.edu.sg** for a complete listing of our faculty and exciting areas of research. A PhD degree and a strong record of research excellence are required.

Stem cell and developmental biology: We have multiple openings for post-doctoral fellows to perform functional genomics on embryonic stem cells and in early embryonic development. The genes and signaling pathways that regulate cell fate decisions are being explored. You will use tools in genomics, cell biology, and developmental biology to dissect the regulatory networks that control cell differentiation and proliferation in vitro and in vivo.

Transcriptional networks: You will use comprehensive approaches to correlate gene expression with the epigenetic status of stem cells. You will learn and apply new chromatin immunoprecipitation technologies to assess transcription factor occupancy across the genome. In addition, you will evaluate histone and DNA modifications to build a more complete picture of the transcriptional regulatory networks that impact stem cell biology. The ideal candidate will have experience and continued interest in applying molecular biology and biochemical approaches to study gene expression.

Human Genetics (Infectious/Autoimmune Disease): You will work with a multidisciplinary human genetics group to explore the underlying molecular basis of the host response to infectious disease, and/or the vulnerability to autoimmunities. Gene targets will be identified from genome-wide and candidate gene association studies that are ongoing at GIS. Your role will be to apply skills in genetic epidemiology, molecular, and/or cell biology to characterize the novel functions of the implicated genes, and to discover their relationship to the disease process.

Systems biology: Projects for qualified individuals are available to explore biology via a systems-based approach. You will mine our proprietary data sets from genomic and genetic studies to identify candidate genes that are involved in human disease. Computational approaches will drive the selection of disease genes, which will then be evaluated by experimental strategies you design. Experimental biologists with basic computational skills are encouraged to apply. Positions are also available for experienced bioinformaticsists interested in gene expression networks, statistical genetics, and comparative genomics.

If you are interested in joining a highly talented research team situated in a unique location with a global vision, please forward a cover letter, curriculum vitae and a list of three references to:

Office of Research Affairs, Genome Institute of Singapore
Genome, 60 Biopolis Street, #02-01, Singapore 138672
Email : gisrecruit@gis.a-star.edu.sg
(Only shortlisted candidates will be notified)

Director Positions

Agricultural Biotechnology Research Center and Research Center for Biodiversity Academia Sinica, Taipei, Taiwan

Academia Sinica, Taipei, Taiwan, invites applications and nominations for the positions of Director of Agricultural Biotechnology Research Center (ABRC) and Director of Research Center for Biodiversity (RCB). The initial appointment is for a period of three years (renewable for a second term), and will also carry the title of Research Fellow.

Academia Sinica is the pre-eminent academic institution in Taiwan. It is devoted to basic and applied research in mathematics and physical sciences, life sciences, and humanities and social sciences. ABRC currently engages in interdisciplinary research in crop plant improvement and plant bioreactors, herbal medicine and vaccine technology. Research efforts of RCB cover all major areas in biodiversity research including taxonomy and systematics, ecology and biomonitoring, evolutionary genomics, and conservation for sustainability. Both Centers are well funded and equipped with modern research facilities. For details about Academia Sinica and the two Centers, please consult the website: **http://www.sinica.edu.tw**.

Interested candidates should have a Ph.D. degree, a distinguished record of academic scholarship, and diverse experience in university and professional service. He/she is expected to pursue a vigorous research program. The successful candidates will be expected to build on the existing strengths of the respective Centers, develop new research thrusts, promote basic life sciences and provide intellectual leadership in relevant basic and applied life sciences in Taiwan.

Applications and nominations, including complete curriculum vitae, a publication list, and three letters of recommendation, should be submitted to: **Dr. Andrew H.-J. Wang, Vice President, Academia Sinica, 128 Academia Road Section 2, Nankang, Taipei, 115, Taiwan**. Please indicate which position you apply for. Screening of applications/nominations will begin immediately, and will continue until the positions are filled.

Chair of Neuroscience

TUFTS UNIVERSITY SCHOOL OF MEDICINE

Tufts University School of Medicine seeks a Chair for the Department of Neuroscience. The Medical School is located in downtown Boston and has an outstanding reputation in research, teaching, graduate training, and patient care at its affiliated teaching hospitals.

The Department of Neuroscience has 6 tenured, 3 tenure-track faculty members and 1 research-track faculty member with substantial extramural research support. Current faculty research and teaching focus on interests ranging from developmental neurobiology to physiology and behavior, with an emphasis on cellular, molecular, and genetic analyses. We have strong research groups in the areas of sensory systems and complex behavior, and synapse development and function. Ongoing research efforts are shedding light on the etiologies of diseases such as epilepsy, mental retardation, schizophrenia, brain cancer, neurodegeneration (e.g., Alzheimer's and Parkinson's diseases), sensory and sleep disorders (e.g., retinal degeneration, deafness, pain, and insomnia), diabetes, obesity, anxiety, aggression, and drug abuse. The Department is the home of an NIH-funded Neuroscience Core Facility supporting Bioinformatics, Imaging (confocal and two photon), Electrophysiology, and Behavior on the Health Sciences campus. The Neuroscience faculty also is strongly linked to several federally funded Tufts centers that provide core support for nucleotide and protein synthesis and sequencing, and for research in gastrointestinal, cardiovascular and ophthalmologic diseases. The departmental laboratories expanded into new research facilities in 2003. The Department's website is: http://neurosci.tufts.edu.

Department of Neuroscience faculty are involved in teaching graduate students and post-doctoral fellows enrolled in the Tufts University Sackler School of Graduate Biomedical Sciences and also teach a highly-rated course in Neuroscience for second year medical students.

Candidates for the position must hold a Ph.D., M.D. or equivalent degree and qualify for professorial appointment. Applicants/nominees should have active, independent research programs, should be recognized scholars and educators in their fields, and be able to lead the further development of programs of the Department. Candidates with a research program relevant to neurological disease, including research integrating disciplines from the mathematical and engineering sciences, are particularly invited to apply.

Names and curricula vitae of potential candidates should be sent to: **Daniel Jay, Ph.D., Chair, Neuroscience Search Committee, c/o Mary Broderick, Medical Dean's Office, Sackler 8, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111; Mary.Broderick@tufts.edu**

Tufts University is an AA/EO employer and actively seeks candidates from diverse backgrounds.

Tufts UNIVERSITY

POSITIONS OPEN

The Department of Microbiology, Molecular Biology, and Biochemistry at the University of Idaho invite applications for a **TENURE-TRACK ACADEMIC FACULTY** position at the **ASSISTANT** level to begin in the fall of 2007. Applicants should have research interests and experience in the molecular basis of microbial pathogenesis. Candidates should have a Ph.D. in microbiology or related discipline, postdoctoral training, ability to communicate effectively with students and colleagues, and a record indicating outstanding abilities and potential in research and teaching. The successful candidate will be expected to develop and maintain an externally funded research program and participate in graduate and undergraduate teaching in the areas of prokaryotic molecular biology and pathogenic microbiology. The Department has an active pathogenics group and BSL-3 facilities. Salary to commensurate with experience.

Candidates should complete the online application at **website: http://www.hr.uidaho.edu.** Submit letter of application, curriculum vitae, statement of current and long-term research interests and teaching philosophy, and copies of significant publications. In addition, we will need three letters of reference from individuals addressing research potential, teaching, and communication skills. Documents that cannot be submitted online are to be sent to:

Dr. Scott A. Minnich, Search Committee Chair
Department of Microbiology, Molecular Biology, and Biochemistry, University of Idaho
P.O. Box 443052, Life Science Building, Room 142
Moscow, ID 83844-3052

Review of applications begins March 1, 2007, and continues until the position is filled. Priority will be given to applications received prior to March 1, 2007. *Applicants who are selected as final possible candidates must be able to show proof of eligibility to be employed in the United States.*

The University of Idaho is an Equal Opportunity/Affirmative Action Educator and Employer.

EPITHELIAL BIOLOGY RESEARCH POSITIONS
University of Chicago

Positions are available beginning February 2007 in a new multi-investigator, interactive Laboratory of Epithelial Pathobiology focused on mechanisms of adhesion and signaling related to epithelial morphogenesis, wound healing, and carcinogenesis; regulation of ion transport; and polarized membrane trafficking and targeting.

Positions are available both for new Ph.D. recipients at the **POSTDOCTORAL FELLOW** level and for more experienced individuals wishing to transition to independence. Appointment of the latter will be as **RESEARCH ASSOCIATE (ASSISTANT PROFESSOR)** with the potential for consideration of subsequent appointment to the faculty tenure track at a later date.

Minimum requirements are a Ph.D.degree, two to three significant publications, and excellent communication skills. Candidates with an M.D. and prior research experience in epithelial pathobiology are also encouraged to apply.

Interested candidates should submit a letter of interest and current curriculum vitae to: **Karl Matlin, Ph.D.** at **e-mail: kmatlin@surgery.bsd.uchicago.edu.** *The University of Chicago and its Medical Center are Affirmative Action/Equal Opportunity Employers.*

The Department of Biology at Valdosta State University in Valdosta, Georgia, is seeking applications for a tenure-track faculty position in physiology at the **ASSISTANT PROFESSOR** level to begin in August 2007. The successful candidate will teach majors and nonmajors introductory biology courses, anatomy and physiology, and other courses in the candidate's area of expertise. Review of applications will begin February 5, 2007, and continue until the position is filled. For full job description and application details visit our **website: http://services.valdosta.edu/databases/jobs/.** *Valdosta State is an Equal Opportunity Education Institution.*

POSITIONS OPEN

USDA ARS Agricultural Research Service
www.ars.usda.gov

RESEARCH LEADER INTERDISCIPLINARY: SUPERVISORY RESEARCH PLANT PATHOLOGIST/PLANT PHYSIOLOGIST/GENETICIST (GS-14/15)
Salary Range of $87,533 to $133,850

The Grain Legume Genetics and Physiology Research Unit, Pullman, Washington, is seeking a permanent full-time Research Leader. The successful candidate will provide scientific leadership and operational management for the research unit and conduct personal research on physiology, pathology, and/or genetics and breeding of cool season food legumes contributing to the development of improved germplasm and cultivars. For details and application directions, see announcement number ARS-X7W-0077 at **website: http://www.afm.ars.usda.gov/divisions/hrd/index.html.** To have a printed copy mailed, call **telephone: 509-335-8663.** *U.S. citizenship is required.* Announcement closes March 5, 2007. Applications must be received by the closing date of the announcement. *USDA/Agricultural Research Service is an Equal Opportunity Employer and Provider.*

GLOBAL VACCINES, INCORPORATED
New Vaccine Technologies for the Developing World

Global Vaccines, Incorporated (GVI) is a not-for-profit company that is translating basic discoveries into affordable vaccines for resource-poor populations. We are currently hiring additional scientists at **STAFF, POSTDOCTORAL,** and **TECHNICAL** levels to join our expanding research team. The successful candidates will have strong virology/microbiology, molecular biology, and immunology skills, as well as the ability to perform and communicate well in a hardworking team environment. Experience with vaccine design and development is preferred but not required. GVI is located in Research Triangle Park, North Carolina, and offers competitive salary and benefit packages. To learn more about GVI please visit us at **website: http://www.globalvaccines.org.** Applicants should submit a cover letter indicating personal qualifications and detailed curriculum vitae to:

Clayton Beard
Director of Research
Global Vaccines, Incorporated
7020 Kit Creek Road, Suite 250
P.O. Box 14827
Research Triangle Park, NC 27709-4827
E-mail: cbeard@globalvaccines.org

ASSISTANT SCIENTIST TERRESTRIAL ECOSYSTEM ECOLOGIST
Marine Biological Laboratory

The Ecosystems Center at the Marine Biological Laboratory (MBL) seeks applications for an Ecosystem Ecologist at the Assistant Scientist level with focus area of land-water interactions and understanding consequences of large-scale changes to terrestrial land use and climate. Of particular interest are individuals capable of working at a variety of spatial scales or who use novel techniques to investigate ecosystem process. The Center has a highly collaborative research environment. The successful applicant should show strong potential to develop an externally funded independent research program in collaboration with other Center scientists and scientists from other institutions. More information about the Center is available at **website: http://www.mbl.edu/research/resident/lab_ecosystem.html.**

For full job posting and application procedure, access the position posting on the MBL jobs page at **website: http://www.mbl.edu/jobs.** Review of applications will begin March 1, 2007.

The MBL is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

MARINE BIOLOGIST

The University of Alabama (UA) Department of Biological Sciences and Dauphin Island Sea Laboratory (DISL) (the State of Alabama's Marine Science Institution) announce a tenurable **ASSISTANT/ASSOCIATE PROFESSOR** Marine Biologist position to begin August 2007. We are seeking a highly interactive scientist who is capable of and willing to conduct collaborative research in freshwater to marine systems, complementing ongoing research at UA and DISL. The successful candidate will have an established record in research, grants, service, and undergraduate and graduate teaching. We invite applications from Estuarine Ecologists/Biologists working at any level of ecological organization and studying areas such as: population genetics; food webs, including microbial loops; nutrient cycling; or physiological ecology. Researchers working on conservation biology, anthropogenic impacts in the coastal zone, and climate change are also encouraged to apply.

Appointees will be expected to develop active, externally funded research programs, to work closely with undergraduate and graduate students as a teacher, advisor, and Director of research, to coordinate our graduate program in marine science, and to serve as a liaison between UA and DISL.

For more information about our programs visit our websites at **website: http://www.as.ua.edu/biology** and **http://www.disl.org/.** To apply, send curriculum vitae and a letter of application that includes (1) your research goals; (2) a statement of your teaching philosophy; and (3) a list of courses in your area of expertise that you would be interested in teaching, and have at least three letters of reference sent to: **Marine Biology Search, Department of Biological Sciences, P.O. Box 870344, The University of Alabama, Tuscaloosa, AL 35487-0344.** Questions about the position may be addressed to: **Dr. Julie Olson, Chair of the Search Committee (e-mail: jolson@biology.as.ua.edu).** Review of applications will begin February 15, 2007, and continue until the position is filled.

The University of Alabama is an Equal Opportunity/Affirmative Action Employer and welcomes applications from women and members of minority groups.

A **POSTDOCTORAL POSITION IN IMMUNOLOGY** is available immediately to investigate the impact of nutritional deficiencies on immune defense systems. Such deficiencies accompany many chronic illnesses and cause increased production of immunosuppressive steroids as part of a stress response. Thus the interrelationships between nutrients, neuroendocrine systems, gene expression, and immune defense are explored in this novel area of research. A resourceful candidate with a background in immunology and molecular biology is desired. This well-funded NIH position provides a generous salary and benefits for two years. The projects developed by a successful candidate can be used to establish their own independent laboratory. Send curriculum vitae, a letter describing career goals, and contact information for three references to: **Dr. Pam Fraker (e-mail: fraker@msu.edu), Department of Biochemistry, Michigan State University, East Lansing, MI 48824.**

A **POSTDOCTORAL POSITION** is available immediately at Texas Tech University Health Science Center. The prospective candidate will work on polycystic kidney disease and kidney cancer at both basic and translational levels. The successful candidate should have a Ph.D. and/or M.D. degree and a strong background in molecular biology. The experience with small animal models is a plus. The candidate must be highly motivated and capable of functioning as a teamplayer. Send curriculum vitae, including research experience, publication list, and names and contact information of three references, to **Dr. Yunxia Tao** by **e-mail: yunxia.tao@ttuhsc.edu.**

OAKLAND UNIVERSITY
DEPARTMENT OF BIOLOGICAL SCIENCES

TENURE-TRACK FACULTY POSITIONS IN MICROBIOLOGY AND IMMUNOLOGY

The Department of Biological Sciences at Oakland University invites applications for two tenure-track positions to be filled by August 2007, one in **Microbiology** and one in **Immunology**. We seek candidates who are interested in key molecular/biochemical questions in their fields, using state of the art approaches and techniques. A Ph.D. and post-doctoral experience are required as well as a strong research track record evidenced by publications. Appointments will be at the assistant professor level; outstanding candidates with appropriate experience and long-term funding may be considered for appointment as associate professor. Each successful candidate is expected to develop a vigorous, extramurally funded research program, to teach effectively at the undergraduate and graduate levels, and to mentor graduate students in doctoral programs.

The Department of Biological Sciences (**http://www2.oakland.edu/biology/**) is a modern, well equipped, and research oriented department. The department has active graduate programs at the Master's and Ph.D. levels. Oakland University is a state-supported institution of 17,000 students situated on a beautiful 1,600-acre campus 25 miles north of Detroit.

Review of Applications will begin on January 15, 2007, and continue until the position is filled. Applicants should submit a curriculum vitae, statement of research plans and teaching philosophy, key reprints, and arrange to have at least three letters of reference sent to:

Arik Dvir, Chair
Department of Biological Sciences
Oakland University
Rochester, MI 48309-4401

Or by email: **biology1@oakland.edu**

Oakland University is an Affirmative Action/Equal Opportunity Employer and encourages applications from women and minorities.

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

STRUCTURAL BIOLOGIST
Johns Hopkins Malaria Research Institute
Department of Biochemistry and Molecular Biology
Department of Molecular Microbiology and Immunology

The Johns Hopkins Malaria Research Institute and the Departments of Biochemistry and Molecular Biology and Molecular Microbiology and Immunology invite applications for a tenure track faculty position in the area of structural biology. Preference will be given to candidates at the assistant professor level, but candidates at more senior levels will be considered. **We particularly seek applicants whose research employs x-ray crystallographic and biophysical approaches to address fundamental questions in microbiology or immunology.**

Extensive core facilities are available, including a biophysics facility with equipment for crystallography, calorimetry and spectroscopy. A competitive start-up package, salary and benefits will be provided. Individuals will be expected to develop independent research programs within an interactive environment of investigators interested in the pathogenesis of viral, bacterial and parasitic diseases and the biochemistry and molecular biology of fundamental cellular processes. Opportunities exist for interaction with the vibrant Johns Hopkins biophysical research community and graduate training programs. Applicants must have a PhD, MD, or equivalent degree and appropriate post-doctoral experience.

Applicants should provide curriculum vitae, description of research interests and three references by **March 15, 2007**. For more information, or to submit an application, please contact: **Susan Booker, JHMRI Coordinator, Johns Hopkins Bloomberg School of Public Health, Room E5132, 615 North Wolfe St, Baltimore, MD, 21205; V. 410-502-3377; F. 410-955-0105; sbooker@jhsph.edu**. For information on JHMRI, BMB and MMI faculty and research programs, please visit **www.jhsph.edu**.

The Johns Hopkins University actively encourages interest from women and minorities and is an Affirmative Action/Equal Opportunity Employer.

THE CANON NATIONAL PARKS SCIENCE SCHOLARS PROGRAM

Training the Next Generation of Conservation Scientists

The Canon National Parks Science Scholars Program is pleased to announce its 2007 competition. The program is a collaboration among Canon, the American Association for the Advancement of Science and the US National Park Service. Thanks to a generous commitment by Canon, the program will be awarding eight US$80,000 scholarships to Ph.D. students throughout the Americas to conduct research critical to conserving the national parks of the region.

Research projects in the biological, physical, social and cultural sciences are eligible, as well as research projects in technology innovation in support of conservation science.

Applications must be received by **3 May 2007**. For information about the Canon National Parks Science Scholars Program and a copy of the application guide, please visit the website **<www.canonscholars.org>**.

Canon

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

UNIVERSITY MEDICAL CENTER
THE UNIVERSITY OF TOLEDO

Faculty Positions
Center for Diabetes and Endocrine Research (CeDER)

The newly established Center for Diabetes and Endocrine Research (CeDER) at the UT College of Medicine invites outstanding scientists with Ph.D., M.D., M.D./Ph.D., or equivalent degrees to apply for faculty positions at the Assistant or Associate Professor level. Appointments will be in the Department of Physiology, Pharmacology, Metabolism and Cardiovascular Sciences with membership in CeDER. Physician scientists will hold joint appointments in an appropriate clinical department.

Candidates are expected to develop, or to have a vigorous, extramurally funded program, which complement existing strengths in diabetes and obesity research. Successful candidates will be expected to participate in medical and graduate education.

Candidates with a track record of funding and research in islet and lipid biology, neuroendocrine regulation of metabolic disorders, molecular aspects of nutrition, and whole animal metabolism are encouraged to apply.

Applicants should submit their curriculum vitae, a brief description of current and future research plans, selected publications, and contact information for at least three references to:

Elizabeth Akeman
Assistant to Dr. Sonia M. Najjar, Director of CeDER
UT College of Medicine
3035 Arlington Ave.
Mail Stop 1008
Toledo, OH 43614-2598
Elizabeth.Akeman@utoledo.edu

UT is an Equal Access, Equal Opportunity, Affirmative Action Employer and Educator.

POSITIONS OPEN

BUDD LARNER, PROFESSIONAL CORPORATION

Budd Larner is one of the oldest and most prestigious full service law firms in New Jersey, with headquarters in Short Hills and satellite offices in New York City, Cherry Hill, New Jersey, and Atlanta, Georgia. We are an Equal Opportunity Employer and offer a competitive compensation and benefits package, a collegial working environment and challenging work.

We currently seek the following seasoned legal professionals for our Short Hills office:

PHARMACEUTICAL PATENT ATTORNEY with two to six years of experience in litigation and/or drafting of noninfringement/invalidity opinions. Experience in the pharmaceutical/chemical field or closely related field preferred. Emphasis on Hatch-Waxman and FDA issues a plus. Candidates should possess strong academic credentials, strong analytical, research, and communication skills. New Jersey Bar admission and/or United States Patent and Trademark Office (USPTO) registration preferred. Advanced degree a plus.

SCIENTIFIC ADVISER or **PATENT AGENT** with at least one year of experience, preferably in the pharmaceutical field or closely related science field. Candidates should possess an advanced science degree with excellent academic credentials and strong written communication skills. USPTO registration preferred, but not necessary depending on experience.

Qualified candidate should send resume and cover letter to:

Attn: Director of Attorney Recruitment
Budd Larner, P.C.
150 John F. Kennedy Parkway
Short Hills, NJ 07078
Fax: 973-379-7734
E-mail: attorney@budd-larner.com
Website: http://www.buddlarner.com

Budd Larner is an Equal Opportunity Employer.

DIRECTOR, DIVISION OF INTEGRATIVE ORGANISMAL BIOLOGY
National Science Foundation, Arlington, Virginia

NSF's Directorate for Biological Sciences seeks candidates for the position of Director, Division of Integrative Organismal Biology (IOB). The Division supports research aimed at integrative understanding of organisms as units of biological organization, with particular emphasis on their development, form, function, and evolution. Information about the Division's activities may be found at **website: http://www.nsf.gov/bio/iob/about.jsp.**

Appointment to this Senior Executive Service position may be on a one to three-year limited term basis, with a salary range of $109,808 to $165,200. Alternatively, the incumbent may be assigned under Intergovernmental Personnel Action (IPA).

Announcement S20070031 with position requirements and application procedures may be obtained on NSF's **website: http://www.nsf.gov/about/career_opps/.** Applicants may also obtain the announcement by contacting the **Executive Personnel Staff** at **telephone: 703-292-8755** (hearing impaired individuals may call **TDD 703-292-8044**). Applications must be received by February 2, 2007.

NSF is an Equal Opportunity Employer.

MOLECULAR BIOLOGY - GENE EXPRESSION

Agarigen Incorporated, an early-stage startup in Research Triangle Park, has openings for three key scientists. Successful candidates will lead a laboratory team developing novel gene expression technology in fungi for biomanufacturing applications. Candidates should possess M.S. or Ph.D. in molecular biology; over two years of experience in fungal or plant gene expression and R&D management; interest in applied research; knowledge of gene expression/regulation; and excellent laboratory skills. Competitive salary/benefits and opportunity to share in the growth of the company. Submit curriculum vitae and letter to **e-mail: recruiting@agarigen.com** or mail to: **Agarigen, 2801 Thoreau Drive, Durham, NC 27703.**

POSITIONS OPEN

EMORY UNIVERSITY

POSTDOCTORAL FELLOW POSITIONS are available in the joint program of **Fadlo R. Khuri, M.D.** and **Haian Fu, Ph.D.** to study molecular oncogenesis and mechanism-based anticancer drug discovery at Emory University School of Medicine's Winship Cancer Institute. Motivated candidates with strong biochemistry, cell biology, chemistry, or pharmacology research backgrounds are encouraged to apply. Emory University is located in a beautiful area in the city of Atlanta.

Interested individuals should send their curriculum vitae and contact information for three references to: **Ms. Judie Wells, Winship Cancer Institute, Emory University, Atlanta, Georgia,** via **e-mail: jwell06@emory.edu.**

Emory University is an Equal Opportunity, Affirmative Action Employer. Women and members of minority groups are strongly encouraged to apply.

ASSISTANT PROFESSOR
Plant Molecular Genetics

The Department of Microbiology, Molecular Biology, and Biochemistry at the University of Idaho seeks to fill an academic year tenure-track position beginning as early as fall 2007. The successful candidate must be capable of establishing a nationally competitive research program in plant molecular biology or plant genetics. We are particularly interested in persons using molecular and genetic techniques to study important basic plant biological questions in the areas of disease resistance, improved nutritional quality, or bioenergy research. Successful candidates for this position must demonstrate the ability to communicate effectively and will be responsible for teaching an annual one-semester lower-division class in molecular biology and biotechnology, and participate in a team-taught upper-division/graduate course in his/her area of expertise to be taught in alternate years. Applicants must have a Ph.D. in molecular biology, biochemistry, genetics, or an appropriately related field and postdoctoral experience with a strong publication record.

Review of applications begins March 1, 2007, and continues until the position is filled. Priority will be given to applications received prior to March 1, 2007. *Applicants who are selected as final possible candidates must be able to show proof of eligibility to be employed in the United States.*

Candidates should complete the online application at **website: http://www.hr.uidaho.edu.** Submit letter of application, curriculum vitae, statement of current and long-term research interests and teaching philosophy and copies of significant publications. In addition, we will need three letters of reference from individuals addressing research potential, teaching and communication skills. Documents that cannot be submitted online are to be sent to:

Plant Molecular Genetics Search Committee
Department of MMBB, University of Idaho
P.O. Box 443052, Life Science Building, Room 142
Moscow, ID 83844-3052

More information about the Department can be found at **website: http://www.ag.uidaho.edu/mmbb/.**

To enrich education through diversity the University of Idaho is an Affirmative Action/Equal Opportunity Employer and Educational Institution.

TWO POSTDOCTORAL FELLOWS, doctoral degree, experience and knowledge of inner-ear anatomy required, electrophysiology and molecular biology competence preferred, application deadline January 8, 2007, or until filled, start dates (negotiable): (1) March 2007; (2) July 2007. Contact **Dr. Daniel Marcus, e-mail: marcus@ksu.edu.** *Kansas State University is an Equal Opportunity Employer.*

POSITIONS OPEN

EMPLOYMENT OPPORTUNITIES
Division of Chemistry
National Science Foundation

The Division of Chemistry of the National Science Foundation (NSF) is pleased to announce the availability of multiple openings for permanent and temporary **PROGRAM OFFICERS** in the different areas (see **website: http://www.nsf.gov/div/index.jsp?div=CHE** for programs and areas). These interesting and rewarding positions provide salary in the range $91,047 to $142,449, commensurate with experience. Alternatively, these positions may be filled under the terms of the Intergovernmental Personnel Act (IPA). Applicants must have a Ph.D. in chemistry or a closely related field, demonstrated expertise in one or more areas covered by the Division of Chemistry, plus six or more years of successful research, research administration, and/or managerial experience pertinent to the position.

Applicants for permanent positions in chemistry should see **website: http://nsf.gov/about/career_opps to apply.** Applications must be received by February 1, 2007.

Appointment to temporary positions may be on a one or two-year visiting scientist appointment, a federal temporary appointment or an IPA. The NSF provides generous support to allow the holders to maintain active research programs at their home institutions. For more information on these appointments please refer to **website: http://www.nsf.gov/pubs/2006/nsf06056/nsf06056.jsp.**

Applicants for temporary assignments should send a letter of interest and curriculum vitae to: **Dr. Luis Echegoyen, Director, Division of Chemistry, National Science Foundation, e-mail: echegoyen@nsf.gov.**

The appointees are expected to work with the chemistry community to broaden the diversity of participants in NSF programs, and to integrate research and education in chemistry.

Please contact **Dr. Echegoyen** for further information.

NSF is an Equal Opportunity Employer committed to employing a highly qualified staff that reflects the diversity of our nation.

RESEARCH SCIENTIST OR RESEARCH ENGINEER
Category: Industry Position
Hitachi Chemical Research Center, Incorporated

Hitachi Chemical Research Center, Incorporated (HCR), a subsidiary of Hitachi Chemical Company, Limited, is located in southern California on the University of California, Irvine campus. HCR is a research and development company directed towards novel technology platforms and related biomaterials for life sciences. The candidate will focus on research in the nanotechnology or nanomaterial fields to develop polymer-based functional materials. The individual will create, develop, and direct his/her own project. Candidate must be an independent researcher who has demonstrated scientific creativity and technical proficiency in his/her field. In addition, the candidate must have a strong background in a wide range of chemistry including biochemistry, preferably working with polymers or in material sciences. Position requires a minimum of a Ph.D. in appropriate science or engineering. HCR offers competitive benefits and salary. Interested candidates can e-mail resumes to **Ms. Lisa Osborn** at **e-mail: losborn@hcrcenter.com.** *Equal Opportunity Employer.*

POSTDOCTORAL POSITION
Department of Pharmaceutical Sciences
University of Pittsburgh

Postdoctoral position available for Ph.D. **BIOCHEMIST/MOLECULAR BIOLOGIST** to study how specific DNA lesions induce toxicity and/or mutations. Preferred experience: Maxam-Gilbert sequencing; DNA amplification and purification, protein purification; cell culture. Send resume, including the names, addresses, and telephone numbers of three references to **Dr. Barry Gold** at **e-mail: goldbi@pitt.edu.** *The University of Pittsburgh is an Affirmative Action/Equal Opportunity Employer.*

Proteomics Core Facility Senior Director will manage all aspects of proteomic technology, development, fabrication, quality control, and distribution of high quality reagents for proteomic research. This person will help guide research projects that use HPLC, GC, MALDI, techniques to characterize the proteome and will lead efforts to devise, adopt, and/or test new technologies. The successful candidate will have a strong background in protein biochemistry and will have prior experience in the use of all the above techniques.

Education: Ph.D. in biochemistry, pharmacology, bioorganic chemistry, molecular biology or related field. Candidates with a Master's degree will also be considered based on experience.

Training/Experience: 5-7 years hands-on research in the area of proteomics or related areas of molecular biology. Academic or industrial experience is acceptable. Also, a track record that demonstrates publications in peer-reviewed journals is preferred. Past experience in a customer-service oriented laboratory/core facility is highly preferred as is experience supervising a High-Throughput screening core facility.

Equipments in the Core: ABI 4800 Proteomics Discovery System with Mascot; Tempo LC MALDI spoting system, HPLC.

Competencies: Ability to plan, organize and coordinate work assignments. Ability to establish and maintain effective working relationships with others as well as communicate verbally and in writing.

Interested candidates must apply online at **http://jobs.texastech.edu**. The position is open until filled. Application review will begin immediately. Please mail a cover letter, curriculum vitae with email address, representative publications, and three letters of reference to:

Dr. Rajkumar Lakshmanaswamy
Chair, Search Committee
Texas Tech University Health Sciences Center
5001 El Paso Drive
El Paso, TX 79905
915-783-5227 (Telephone)
915-783-5222 (Fax)
rajkumar.lakshmanaswamy@ttuhsc.edu

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER IS AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER. WOMEN AND MINORITIES ARE ENCOURAGED TO APPLY.

The Genomics Core Facility Director will manage all aspects of microarray development, fabrication, quality control, and distribution of high quality reagents for genomic research. This person will help guide research projects that use large-scale cDNA libraries and microarrays to characterize genomes and will lead efforts to devise, adopt, and/or test new technologies, e.g. on-slide oligonucleotide synthesis and novel imaging techniques. The successful candidate will have a strong background in biochemistry and will have prior experience in the use of microarrays and robotics.

Education: Master's degree in biochemistry, pharmacology, bioorganic chemistry, molecular biology or related field. Candidates with a Ph.D. degree in one of the above mentioned fields will be given preference.
Training/Experience: 5-7 years hands-on research in the area of genomics or related areas of molecular biology. Academic or industrial experience is acceptable. Also, a track record that demonstrates publications in peer-reviewed journals is preferred. Past experience in a customer-service oriented laboratory/core facility is highly preferred as is experience supervising a High-Throughput screening core facility.
Equipments in the Core: ABI PRISM 7900HT Sequence Detection System with TaqMan Low Density Array upgrade, ABI 1700 Microarray Chemiluminescent Scanner, AB 3130x16 Capillary Genetic Analyzer.
Competencies: Ability to plan, organize and coordinate work assignments. Ability to establish and maintain effective working relationships with others as well as communicate verbally and in writing.

Interested candidates must apply online at **http://jobs.texastech.edu**. The position is open until filled. Application review will begin immediately. Please mail a cover letter, curriculum vitae with email address, representative publications, and three letters of reference to:

Dr. Rajkumar Lakshmanaswamy
Chair, Search Committee
Texas Tech University Health Sciences Center
5001 El Paso Drive
El Paso, TX 79905
915-783-5227 (Telephone)
915-783-5222 (Fax)
rajkumar.lakshmanaswamy@ttuhsc.edu

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER IS AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER. WOMEN AND MINORITIES ARE ENCOURAGED TO APPLY.

THE OHIO STATE UNIVERSITY Medical Center

The **Department of Molecular and Cellular Biochemistry** of **The Ohio State University** invites applications for tenure track positions at any level of appointment, including **Assistant, Associate** and **Full Professor**. Appointment at the Assistant Professor level requires a minimum of 3 years of postdoctoral experience backed by publications in established journals and the potential to generate research support. Senior level candidates should have established, well-funded research programs. We are particularly interested in candidates who are exploring the biochemical and molecular aspects of disease processes. Columbus has been ranked by *Money* as one of the top ten cities in the country. Faculty in the Department have access to graduate students from a large pool of interdisciplinary graduate programs. An attractive start-up package including modern laboratory space will be available.

Applicants should submit a *curriculum vitae*, selected reprints, a description of research interests and goals, grant support, and the names, addresses and telephone/fax numbers of three references to: **Chair, Faculty Search Committee, The Ohio State University, College of Medicine, Department of Molecular and Cellular Biochemistry, 333 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210-1218**.

POSITIONS OPEN

TWO POSTDOCTORAL POSITIONS available immediately in the Department of Chemistry and Biochemistry of the University of Maryland, Baltimore County (UMBC).

One position will focus on the development of methods for structural characterization of RNA-protein macromolecular complexes based on state-of-the-art high-resolution mass spectrometry. A strong background in mass spectrometry is required. If necessary, training in biochemistry and molecular biology will be provided. Available instrumentation includes a 7T and a 12T Fourier transform ion cyclotron resonance mass spectrometry system.

The other will focus on the development of methods for molecular modeling of proteins and protein-RNA complexes from sparse distance constraints. The ideal candidate should have experience in different areas of computational biology/chemistry, including model building, homology modeling, virtual ligand screening and docking. Available tools include a Linux cluster and an SGI graphic workstation including macromolecular crystallography packages, such as X-PLOR, CNS, and others.

Send your curriculum vitae and the names of two professional references to:

Dr. Dan Fabris
Department of Chemistry and Biochemistry
University of Maryland, Baltimore County
1000 Hilltop Circle
Baltimore, MD 21250
E-mail: fabris@umbc.edu

Applications will be received until a suitable candidate is found for each position.

UMBC is an Equal Opportunity/Affirmative Action Employer and applications from women, minorities, and individuals with disabilities are especially encouraged.

CELL BIOLOGIST

The Biology Department of Franklin and Marshall College invites applications for a three-year **VISITING ASSISTANT PROFESSOR** position, starting July 2007. Candidates should have a Ph.D., demonstrated strength in teaching and research, and ability to engage undergraduates in research. Teaching responsibilities include lecture and laboratory sections of a sophomore-level core course in cell biology and an upper-level elective in the area of specialization. The successful candidate may occasionally contribute to the general education curriculum. Franklin and Marshall College has a tradition of excellence in science and student research; a new life sciences building will open in summer 2007. In addition to the biology major, we offer interdisciplinary majors in biochemistry and molecular biology and in biological foundations of behavior (neuroscience and animal behavior). Applicants should arrange to have three letters of reference sent and should submit curriculum vitae, plans for actively engaging undergraduates through teaching and research, and undergraduate and graduate transcripts. Priority will be given to completed applications received by February 15, 2007. Electronic submissions will not be accepted. Send applications to: **Professor Carl Pike, Department of Biology, Franklin and Marshall College, Lancaster, PA 17604-3003. Telephone: 717-291-4118; fax: 717-358-4548; e-mail: cindy.mcintyre@fandm.edu; website: http://www.fandm.edu/biology.xml**

Franklin and Marshall College is a highly selective liberal arts college with a demonstrated commitment to cultural pluralism. Equal Opportunity Employer.

A **POSTDOCTORAL** position is available immediately to study the host immune response to enteric pathogens in the Division of Infectious Diseases at the Department of Biomedical Sciences, Tufts University Cummings School of Veterinary Medicine. Candidates with a Ph.D. degree or equivalent with experience in cellular and molecular biology are encouraged to apply. Please send curriculum vitae and a list of three references to: **Dr. Saul Tzipori (e-mail: saul.tzipori@tufts.edu)** or **Dr. Hanping Feng (e-mail: hanping.feng@tufts.edu).** *Tufts University is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

DIRECTOR OF RESEARCH TRAINING PROGRAMS

National Multiple Sclerosis Society in midtown seeks an individual to manage fellowship funding programs and other research activities. Will have heavy interaction with professional and lay communities.

Requires Ph.D. in a biomedical science, minimum of three years of experience in laboratory/clinical research or research administration and superior communications and presentation skills. Microsoft Office and web-based program knowledge a must.

Excellent benefits. Fax resume with salary requirements to **fax: 212-476-0535** or **e-mail: hrnyc@nmss.org. Website: http://www.nationalmssociety.org.** Only candidates selected for further consideration will be contacted. *Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.*

POSTDOCTORAL POSITIONS
Molecular Cell Biology of Diabetic Complications

As reviewed in *Nature* **414:**813, 2001, our laboratory focuses on the mechanisms by which hyperglycemia causes vascular damage. We are currently investigating (a) the molecular basis for "metabolic imprinting," (b) the genetic basis for familiar clustering of susceptibility to hyperglycemic damage, (c) endothelial progenitor cell dysfunction and impaired vasculogenesis in diabetes, and (d) identification of novel therapeutic strategies for preventing metabolite-induced vascular damage. Candidates should have a strong foundation in molecular and cell biology. Please send curriculum vitae and names/contact information of three references to:

Dr. M. Brownlee
Diabetes Research Center
Albert Einstein College of Medicine
Jack and Pearl Resnick Campus
1300 Morris Park Avenue
Bronx, NY 10461
E-mail: brownlee@aecom.yu.edu

Equal Opportunity Employer.

The Institute of Zoology, National Taiwan University, is seeking an outstanding individual to fill a full-time faculty position which will be jointly appointed with the Department of Life Science starting on August 1, 2007. The level is for **ASSISTANT PROFESSOR** or higher ranks. The applicant's research area should be related to genomics and bioinformatics, immunology, or physiology. Candidates must have a Ph.D. degree (preferably with postdoctoral experiences). Applicants must submit a photocopy of Ph.D. diploma, curriculum vitae, a list of publications in the past three years, a statement of research and teaching, and three letters of recommendation before January 31, 2007, to: **Chair, Faculty Search Committee, Institute of Zoology, National Taiwan University, Number 1 Section 4, Roosevelt Road, Taipei, Taiwan 10617, e-mail: zoology@ntu.edu.tw.**

POSTDOCTORAL FELLOWSHIP in the Section of Atherosclerosis, Department of Medicine, Baylor College of Medicine. Qualified applicants should have a Ph.D. or M.D. and experience in molecular biology with an interest in lipoproteins, inflammation, obesity, and vascular biology. Highly competitive salary will be offered and is negotiable depending upon experience. Eligibility for NIH training-grant position with *U.S. citizenship or residency required.* Reply with curriculum vitae and three references to: **Christie M. Ballantyne, M.D., Fellow of the American College of Cardiology, Professor, 6565 Fannin, M.S. A601, Houston, TX 77030. E-mail: cmb@bcm.tmc.edu.** *Baylor College of Medicine is an Equal Opportunity/Equal Access/Affirmative Action Employer.*

POSITIONS OPEN

NIH POSTDOCTORAL TRAINING
in Molecular Therapy
The Children's Hospital of Philadelphia/
University of Pennsylvania

National Heart Lung and Blood Institute Training Grant, Training in Molecular Therapeutics for Pediatric Cardiology, supporting studies of cardiovascular disease mechanisms and molecular therapies. Project fields include: cardiac and pulmonary development, heart valve disease, regenerative medicine, stem cell biology, and pharmacology. Competitive salary and full benefits. Only applicants with Ph.D. and/or M.D. *(who must be a resident alien or U.S. citizen status)* should send their curriculum vitae and the names of three references to:

Robert J. Levy, M.D.
William J. Rashkind Endowed Chair
University of Pennsylvania School of Medicine
Training Program Director
The Children's Hospital of Philadelphia, 702 ARC
3615 Civic Center Boulevard
Philadelphia, PA 19104
Fax: 215-590-5454
E-mail: levyr@e-mail.chop.edu.

Equal Opportunity Employer. Minorities/Females/Persons with Disabilities/Veterans.

POSTDOCTORAL POSITIONS for G protein-coupled receptor (GPCR) structure-based drug screening research through nuclear magnetic resonance (NMR), computational and high throughput screening (HTS) approaches. Our laboratory is situated in a new state-of-the-art research building (Biomedical Science Tower Three) where 900, 800, and 700 NMR systems, high computing facility/drug discovery software, and HTS/HCS facilities are available. We are recruiting two positions: (1) **CHEMINFORMATICS COMPUTATIONAL CHEMIST POSITION.** Qualifications: three to five years of working experience in database development and administration using SQL or Oracle, and ASP, C++, VB, or Java web programming. Experience in cheminformatics, computer drug design, and virtual drug screening is plus. (2) **PROTEIN BIOPHYSICS POSITION** for GPCR protein NMR structure biology research. Experience with membrane protein expression/purification and biological function studies is a plus. Salary will be commensurate with experience. Please e-mail curriculum vitae and three names of references to **e-mail: xix15@pitt.edu, Professor XiangQun (Sean) Xie, University of Pittsburgh, website: http://www.pharmacy.pitt.edu, telephone: 412-383-5276.** *The University of Pittsburgh is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION available March 1, 2007, to study molecular mechanisms of lineage specification during early thymocyte development. Available projects include: (1) regulation of thymocyte development by Egr transcription factors; (2) the role of signal strength in the alpha-beta/gamma-delta lineage commitment (*Immunity* **22:** 595-606, 2005); and (3) blockade of Tcell development by a ribosomal protein deficiency. In addressing these questions we employ a variety of in vitro and in vivo models in conjunction with retroviral delivery of candidate genes and shRNA. The successful applicant will enjoy a competitive salary and have access to subsidized housing and childcare, and an active postdoctoral association. Curriculum vitae and three references should be sent to: **David L. Wiest, Ph.D., Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111-2497. Fax: 215-728-2412. E-mail: dl_wiest@fccc.edu.** *Equal Opportunity Employer.*

POSTDOCTORAL TRAINEES in translational immunology at University of California, Los Angeles. *U.S. citizens/permanent residents only.* Ph.D./M.D. with interest in autoimmune diseases and immunology are encouraged to apply. Earliest joining date is April 2007. Salaries per NIH guidelines. Please send your curriculum vitae, research statement, and name, telephone/e-mail of three references to: **Ram Raj Singh, M.D., Department of Medicine/Pathology (e-mail: rrsingh@mednet.ucla.edu).**

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- Grant information
- Resume/CV Database
- Career Forum

ScienceCareers.org
We know science AAAS

POSITIONS OPEN

POSTDOCTORAL POSITIONS: THERMOREGULATION AND INFLAMMATION

We are looking for **POSTDOCTORAL FELLOWS/RESEARCH ASSOCIATES** to study lipid mediators of fever and hypothermia in systemic inflammation, physiological roles of transient receptor potential channels, and behavioral thermoregulation in rats and mice. Background in systems physiology, molecular biology, or immunohistochemistry/neuroanatomy is preferred, but the ability to think and work independently, dedication to work, and persistence in the face of failure are more important than the area of specialization. A specific direction of research will be determined by the Laboratory director to closely match the line of expertise and interests of each successful candidate. Mandatory requirements include an advanced degree, a track record of peer-reviewed publications, excellent computer skills, and good writing skills. Recent publications from the Laboratory include *PLoS Biol* **4:** e284, 2006 and *PLoS ONE* **1:** e1, 2006. Mail your curriculum vitae, reprints of full-length papers, a brief description of research interests and career goals, and names, e-mail addresses, and telephone numbers of at least two references to: **Andrej A. Romanovsky, M.D., Ph.D., Director, Systemic Inflammation Laboratory, Saint Joseph's Hospital, 350 W. Thomas Road, Phoenix, AZ 85013 U.S.A.** *Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITIONS are immediately available in our internationally known research group, which is focused toward understanding the molecular, cellular, and physiological principles of nuclear receptor signaling in normal target cells and endocrine cancers. The successful applicant will utilize state-of-art technologies (i.e. established mouse models, cell-culture, microarray, and proteomics) to address important questions concerning nuclear receptor and coactivator function in normal physiological processes and disease states, such as cancer. Applicants with a strong background in molecular biology and in using animal systems are encouraged to apply. Salaries will be at the upper-competitive end of the national norm. Applicants should have received their Ph.D. and/or M.D. within the past one to two years and, if necessary, *be eligible for an appropriate U.S. visa.* Current curriculum vitae, a brief statement describing prior research and future research goals, and three letters of recommendation (all required before interview) should be sent to: **Dr. Bert W. O'Malley** or **Dr. John P. Lydon, Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030-3498 U.S.A.; fax: 713-798-5599;** or **e-mail: jlydon@bcm.tmc.edu.**

PROFESSOR OF THE PRACTICE

The Department of Ecology and Evolutionary Biology, Tulane University, invites applications for a full-time teaching position. Professors of the Practice are appointed for renewable three-year terms, which include benefits but do not lead to tenure. Candidates must hold a Ph.D. degree and should have teaching experience at the college level. We seek an individual with demonstrated expertise in one or more areas of ecology, evolution, and organismal biology as well as a commitment to excellence in undergraduate instruction, the advancement of science literacy, and the scholarship of teaching and learning. For more details see **website: http://www.tulane.edu/~ebio/News/profprac.htm.** Send curriculum vitae, description of scholarly and teaching interests and experience, selected publications, statement of teaching philosophy, and the names and addresses of three references to: **PoP Search, Department of Ecology and Evolutionary Biology, 310 Dinwiddie Hall, Tulane University, New Orleans, LA 70118-5698.** Review of applications will begin March 1, 2007, and the search will remain open until the position is filled. *Tulane University is an Affirmative Action/Equal Employment Opportunity Employer.*

POSITIONS OPEN

CALIFORNIA INSTITUTE OF TECHNOLOGY
Broad Fellows Program in Brain Circuitry

The California Institute of Technology is looking for a few outstanding scientists from any relevant backgrounds to study how networks of neurons give rise to perception, memory, emotion, and behavior. We encourage applications from individuals employing genetic manipulations in relevant animal model systems, electrophysiological recordings, functional imaging, and computational analyses and related tools. Broad Fellows are independent researchers who have recently received their Ph.D. They will receive internal funding for a group of up to three people. The initial appointment is for three years, with the possibility of renewal for two more years. Excellent salary and benefits. Applications should include curriculum vitae, a statement of research plan, and three letters of recommendation. This material should be submitted online at **website: http://www.broadfellows.caltech.edu** or by **e-mail: heather@klab.caltech.edu.**

Caltech is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.

FACULTY POSITION IN ECOSYSTEM/COMMUNITY ECOLOGY
Wichita State University

The Department of Biological Sciences at Wichita State University (WSU) seeks an Ecosystem/Community **ECOLOGIST** for a tenure-track position at the rank of **ASSISTANT PROFESSOR.** Preferred research areas include, but are not limited to biogeochemical cycling, bioremediation, restoration, and pathogen ecology in the context of global change. The successful candidate is expected to maintain an extramurally funded research program that trains graduate and undergraduate students and utilizes the unique WSU prairie field station. All members of the faculty participate in general biology and nonmajors courses, while offering advanced courses in their discipline. Candidates must have a Ph.D. in the life sciences, postdoctoral experience, and a record of research productivity. Applications must include statements of research and teaching interests, comprehensive curriculum vitae, three sample publications, a list of startup needs with estimates of equipment costs, and contact information for three professional references. We seek applicants who are motivated to work in a collegial atmosphere to foster teaching and research collaborations with existing faculty. The Department includes core facilities in environmental biology, imaging, and bioinformatics, and it maintains an animal care facility and a greenhouse. An attractive startup package is supported by funding from Kansas NSF Experimental Program to Stimulate Competitive Research. More information about the Department is available at **website: http://www.wichita.edu/biology.** Wichita State University is a metropolitan, research-extensive University set in a suburban area of the largest city in Kansas and serves a diverse student body. To insure full consideration, a complete application package must be received by February 12, 2007. However, applications will be considered until the position is filled. Send applications to: **Dr. William J. Hendry, Department of Biological Sciences, Wichita State University, 1845 Fairmount, Wichita, KS 67260-0026.** *Wichita State University is an Equal Opportunity Employer.*

POSTDOCTORAL FELLOW
Harvard Medical School

To study the role of heat shock proteins in tumor immunity. Applicants must have Ph.D. or M.D., and skills including immunology, molecular/cellular biology. Contact: **Dr. S.K. Calderwood, Beth Israel Deaconess Medical Center, 21027 Burlington Avenue, Boston, MA 02215; telephone: 617-632-0628; e-mail: scalderw@bidmc.harvard.edu.**

POSITIONS OPEN

FACULTY POSITION IN MOLECULAR MICROBIOLOGY
Washington University School of Medicine in Saint Louis

The Department of Molecular Microbiology seeks outstanding scientists for a tenure-track faculty position at the **ASSISTANT PROFESSOR** level; senior applicants will also be considered. Areas of particular interest include global infectious diseases, especially protozoan parasites or tuberculosis. We are seeking interactive individuals with a commitment to teaching and the establishment of a vigorous, cutting-edge, independent research program emphasizing fundamental aspects of microbial pathogenesis. Additional information on our Department and program can be found at **website: http://www.microbiology.wustl.edu.** Applicants should send detailed curriculum vitae, selected reprints, description of current and planned research, and arrange to have three letters of reference sent to: **Dr. L. David Sibley, Chair of Molecular Microbiology Faculty Search Committee, Washington University School of Medicine, Campus Box 8230, 660 S. Euclid Avenue, Saint Louis, MO 63110.** Applications may also be sent as a single, merged PDF file (applicant's name as the file name) with letters sent separately to **e-mail: microsearch@borcim.wustl.edu.** The application deadline is March 1, 2007. *Washington University School of Medicine is an Equal Opportunity/Affirmative Action Employer. Women and minorities are especially encouraged to apply.*

INSECT SYSTEMATIST
Illinois Natural History Survey

ASSISTANT PROFESSIONAL SCIENTIST, Insect Systematist. Conduct innovative collection-based research on insect systematics (broadly defined) with relevance to midwestern United States. Requires Ph.D. in entomology or related discipline. Responsibilities: develop vigorous, externally funded research program; publish research findings in scientific journals; curate Illinois Natural History Survey (INHS) insect collection; work with state and federal agencies and University of Illinois. INHS is part of the Illinois Department of Natural Resources and an affiliated agency of the University of Illinois at Urbana-Champaign. To apply send cover letter, curriculum vitae, statement of research interests, and three letters of reference to: **Human Resources Office, PRF# 1479, Illinois Natural History Survey, 1816 S. Oak Street, Champaign, IL 61820. Telephone: 217-265-5644, fax: 217-333-4949, e-mail: hroffice@inhs.uiuc.edu.** Deadline: February 16, 2007. For application requirements and complete position description visit our **website: http://www.inhs.uiuc.edu/opportunities.**

The Case Cardiovascular Research Institute, Division of Cardiovascular Medicine at Case Medical Center invite applications for faculty positions at the level of **ASSISTANT/ASSOCIATE/FULL PROFESSOR.** Candidates should have an M.D., Ph.D., or equivalent degree, an established research program, and track record of independent research funding. Areas of interest include (but not limited to) genetic/epigenetic regulation of cardiovascular cell development, stem cell/regenerative medicine, and human genetics. Case Medical Center offers excellent compensation and benefits package commensurate with applicant's training and experience. Please send curriculum vitae, a statement of current research future research interests, and three letters of support to: **Matt Fletcher, Grants Manager, telephone: 216-368-3980, fax: 368-2951, e-mail: mcf15@case.edu** or to: **Mukesh K. Jain, M.D., Director of Case Cardiovascular Research Institute, The Iris S. and Bert L. Wolstein Research Building, 2103 Cornell Road, Room 4537, Cleveland, OH 44106.**

AWARDS

THE ROYAL SOCIETY

INSTITUT DE FRANCE Académie des sciences

Microsoft Research

The Royal Society and Académie des Sciences Microsoft European Science Award

€250,000

Invitation to nominate

Closing date 21 February 2007

This international award, sponsored by Microsoft Research, is open to any research scientist working in Europe who has made a significant contribution at the intersection of computing and the physical sciences, including mathematics and engineering.

The award will be €250,000, of which €7,500 will constitute prize money with the rest earmarked for further research.

For full details of the award and the online nomination forms please visit **www.royalsoc.ac.uk/microsoft**

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THE ROYAL SOCIETY

Pfizer

Science in Africa – £65,000 available

The Royal Society Pfizer Award

Invitation to nominate
Closing date 21 February 2007

The award is open to any research scientist, working in Africa, whose work in the biological sciences has led to a sustainable positive impact on Africa.

The award will give up to £60,000 to carry out a research project and £5,000 as a prize to the recipient.

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For full details of the award and the online nomination forms visit **www.royalsoc.ac.uk/pfizer**

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Allan L. Goldstein and Enrico Garaci

Confirmed Speakers:

D. Safer, H. Yin, E. Yarmola, P. Riley, R. Dominguez, J. Gomez-Marquez, D. Merlo, W. Sun, B. Zetter, T. Moody, B. Spangelo, L. Larsson, F. Belardelli, L. Romani, W.M. Chu, C. Cierniewski, M. Rojkind, J. Hadden, P. Naylor, H. Kleinman, G. Sosne, C.M. Huang, D. Srivistava, G. Rasi, C. Tuthill, P. Carminati, D. Crockford, J-D. Fine, B. Rubin, E. Hannappel, C. Favalli, A. Mastino, T. Crow, A. Mosoian, A. Mastino, G. Guarnera, M-F. Carlier, H. Yin, R. Robinson, P. Gutowski

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Organisers: Robert Sinclair, **sinclair@oist.jp**
Klaus Stiefel, **stiefel@oist.jp**

Confirmed Speakers:

Gunther Uhlmann, University of Washington
Martin Kreitman, The University of Chicago
Andreas Dress, ICB Shanghai
Hans-Christian Hege, Zuse Institute Berlin
Bob Anderssen, CSIRO Australia
Wayne Rossman, Kobe University
Klaus Stiefel, Okinawa Institute of Science and Technology
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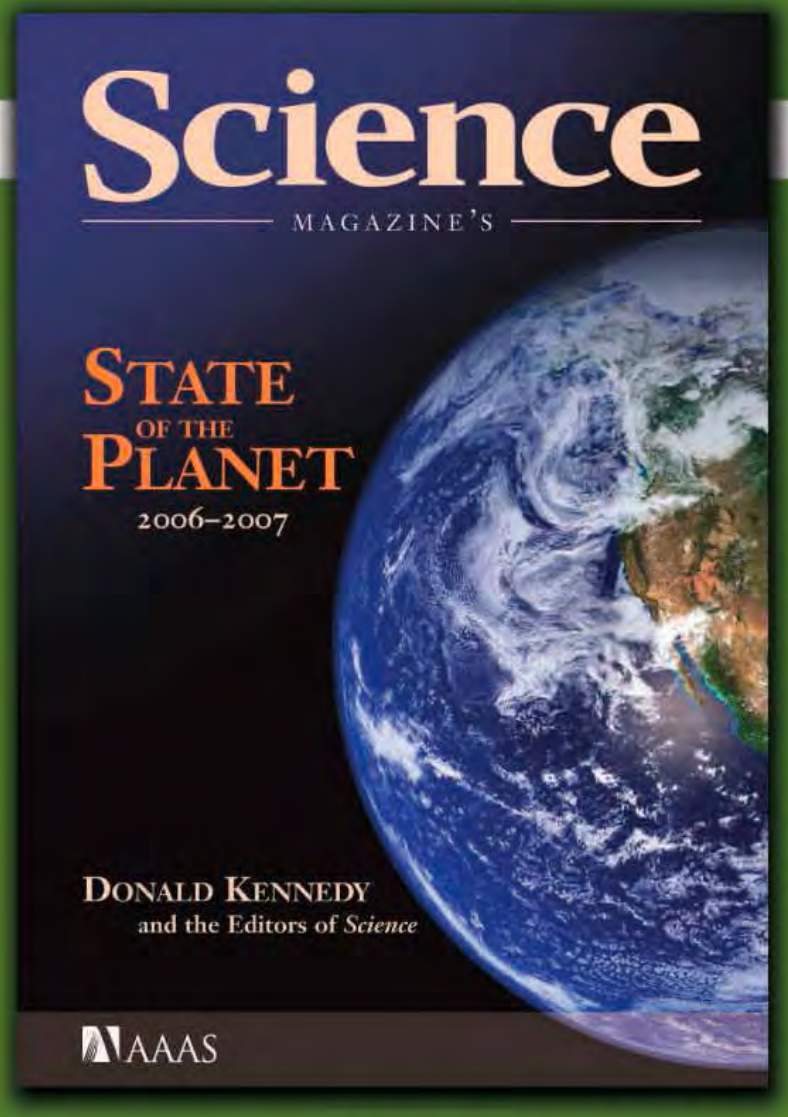

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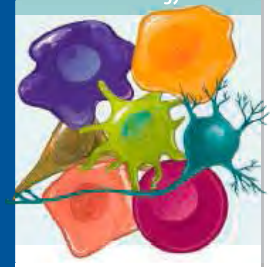

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